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FIFTY YEARS OF GENETICS[1]

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WHEN the baby was born fifty years ago whose development into a giant some of us have witnessed, no proud parents hailed the new addition to the biological family, no relatives surrounding the crib heaped praise and presents upon the child. Quite the contrary: everybody looked askance at the odd creature which did not seem at all to fit into the respectable family. How could such an unimportant thing as purple and white pea flowers impart decisive knowledge on heredity, which, everybody knew, was a phenomenon embracing the entire organism? Who could believe that the playing with numbers and symbols could lead to anything but oddities? Everybody was convinced that the offspring of two different parents was a halfbreed and their offspring a quarterbreed. There was just no room for the strange story of the pea flowers. It seems incredible to us now how few zoologists and botanists realized that a turning point in the study of heredity had arrived and how fewer still were sufficiently impressed as to start, at once, work on those lines.

There were other obstacles to quick success. Among the rediscoverers of Mendel's laws, the already most famous one, de Vries,[2] was more interested in the aberrant behavior

[1] This address was prepared to be read at the opening of the Golden Jubilee Meeting of the Genetics Society of America held at Columbus, Ohio, September 11–14, 1950. When the author learned that this session would be held in common with other biological societies, he substituted a paper on a more general topic. Members of the program committee who had read the present address suggested that it should be published.

[2] As it is impossible to quote all geneticists who made important discoveries, we have restricted quotation of names to the early pioneers and only a few others who are no longer among the living.

of his Oenothera "mutants" than in the simple facts of Mendelian heredity, which were only a side issue with him and which he subsequently played down. Tschermak was engaged in agricultural and horticultural plant breeding, which did not lend him much authority with zoologists and botanists, as things were. Correns, who had gone farthest in the new line of work and followed it up vigorously, happened to be a difficult and queer personality, not the man to impress and convince his reluctant fellow botanists. He worked at the time in the laboratory of the great plant physiologist, Pfeffer. When he asked his boss for a small plot of land in the Botanical Garden in which to continue his experiments, Pfeffer refused, saying that this was not scientific botany but just gardening, which could not be done in his institute. Thus, the important results which Correns accomplished in the following years, working his own garden plot with no help but his wife's, remained largely unknown to his fellow botanists. He had no students or collaborators and grew to middle age before the hostile profession gave him a small professorship.

In view of such a situation it was a lucky event for the young science that a man of unusual energy, endowed with strong fists and a fighting spirit, William Bateson, had already embarked upon what was to become genetical work, again in the face of hostile university colleagues, but backed by a committee of the Royal Society. Thus, with a group of enthusiastic collaborators, he was at once prepared to start Mendelian experiments with both animals and plants. He was soon followed by a few men in different countries, some of whom had also to pay for entering an unpopular line of work. Cuénot in France quickly became one of the founding fathers, which did him little good in the eyes of the scientific powers-that-be in Paris. Toyama in Japan started his pioneer work on silkworms with the result that ten years later I found him past middle age and still an assistant professor. Standfuss and Lang in Switzerland and Johannsen in Denmark, Baur in Germany, Castle, Davenport and Shull in this country and Nilsson-Ehle in Sweden,

soon joined the small group, of whom the last named had to wage a hard uphill fight to find success. After the middle of the first decade, genetics began to become more or less respectable, and at the end of the first decade, was established as an up-and-coming branch of biology.

This did not prevent many zoologists and botanists and still more physiologists and paleontologists from deriding the young science, claiming that Mendelian heredity was only an oddity seen in some unimportant features of the organism. This is the more surprising because at the time of the rediscovery of Mendel's laws many facts were generally known which should have prepared the way. Weismann, in the eighties, had dealt a death blow to the Lamarckian doctrine; Roux and Weismann had shown in the most convincing way that the facts of cytology demanded that the chromosomes be the carriers of the determinants of heredity, and that these must be arranged in a linear order. Furthermore, at an early period, the occurrence of the meiotic divisions had been predicted by Weismann and his prophecies were promptly confirmed. Van Beneden and Boveri had almost completed the basic cytological picture, and Boveri had already started some of the experiments which were to furnish proof of the chromosome theory of heredity. Actually Boveri and Sutton had shown in the first years of Mendelism that the new laws are completely explained by the location of the unit factors, later called genes, in the chromosomes and by the maneuvers of maternal and paternal chromosomes in the meiotic divisions. At about the same time it was realized that the enigmatic accessory chromosomes control the distribution of the sexes among the offspring. Thus, as early as three years after the rediscovery of Mendel, genetics might have assumed her proper place in biology.

That this took much longer is due, at least in part, to the unfortunate circumstance that Bateson, the man who exercised the greatest influence upon the development of genetics in its childhood, just did not believe in chromosomes (and probably did not know much about them). He actu-

ally authored the slogan that genetics must do its work without taking notice of eventual carriers of Mendelian unit factors. Johannsen, the greatest influence in the following years, concurred in this opinion. The leading continental geneticist, Correns, did not exactly share these views and was even willing to accept the role of the chromosomes. However, in a queer kind of jealousy, he always insisted that the decisive facts were the genetical ones which might find interesting but unnecessary confirmation from cytology. Thus, early genetics worked exclusively with the symbolic formulae without the benefit of visualizing their workings in terms of chromosomes. For some reason, which is difficult to understand, even many good biologists were unable to handle this symbolism and therefore derided it. Strange as it sounds, Hans Spemann once told me after a genetical lecture that he never was able or would be able to work those notations. Still, at the end of the first decade when I communicated to a meeting of the German zoologists a simple case of dominant sex-linked heredity which I had found in the nun moth, I was reprimanded by my elders for presenting what nobody in the audience could possibly understand. It is rather fortunate for the development of genetics that (at that time) those who were unable to understand the new science did not have the backing of authoritarian rulers who might have been willing to declare their ignorance the law of the land.

The result of all this was that genetics had to fight for a long time against the belief that it indulged in a half mystical juggling of symbols which, in each case, would be arranged so as to give the desired results. As late as 1906, Morgan himself expressed such a view and still discussed whether the purity of the gametes is proven, and I must plead guilty to having opposed, at that time, the demonstration of the parallel synapsis of homologous chromosomes. Needless to say, for a long time no chairs or lecture courses on genetics were found in the universities of the world.

In spite of all these handicaps to clear sailing and the

small size of the crew steering the genetics ship through sometimes turbulent waters, the accomplishments in the first decade laid the solid basis for the subsequent phenomenal rise of genetics. The universal occurrence of Mendelian heredity was demonstrated in plants, animals and man for all types of visible characters which lent themselves to analysis. Apparently aberrant cases were brought into line, mostly by the work of the Bateson group, including Punnett, Saunders, Doncaster, and of Cuénot; unexpected ratios were explained on Mendelian principles, the interaction of factors with their sometimes strange consequences was elucidated; dominance, intermediacy and epistasis were separated; in a general way what is still today elementary Mendelism was firmly established. Some special cases, such as sex-linked inheritance, were completely described in genetic terms, and the difference between male and female heterogamety understood. The first experiment designed to prove that sex determination acts like a Mendelian backcross (an idea already mentioned by Mendel) was performed by Correns. Simultaneously factorial mutation—as distinguished from de Vriesian mutation—became recognized as the basic fact of genetics, and the first idea towards its explanation was presented in Bateson's presence-absence theory. The first links with neighboring sciences were forged when human traits were found to mendelize, and both medicine and anthropology started, hesitatingly, to incorporate the new knowledge. Eugenics began to enter its first rather primitive Mendelian phase. The discovery that characters of crop plants and domestic animals showed Mendelian behavior started the genetical work in agriculture. Even the first genetic discoveries in yeast were made by Hansen, and Blakeslee inaugurated the genetic work in fungi. The omnipresence of factorial mutation and such discoveries as the Mendelian explanation of atavism secured to the new science its position in the study of evolution.

Next to the firm establishment of the Mendelian doctrine the most far-reaching advance in the first decade was Johannsen's statement of the pure line concept and its

analysis. Here for the first time the difference between non-hereditary modification and hereditary change of unit factors (termed by Johannsen *genes* in abbreviation of de Vries' pangenes) was laid down for all time. The old riddle of variation which Darwin was unable to solve and which his successors, the biometricians, had beclouded still more was solved. Darwin's cautious statement, that all organisms do vary and that some of these variations " tend to be inherited " was replaced by an unequivocal analysis of the types of variation. The relation of phenotype and genotype was established. The bitter feud between the Mendelians and the biometricians, fought especially in England, was ended by the demonstration that the biometric results were what is to be expected on the basis of Mendelian genetics when genetically mixed populations are measured. One of the direct consequences of Johannsen's work, Woltereck's definition of the genotype as a norm of reaction, cleared (or should have cleared) the meaning of the relative roles of heredity and environment. The introduction by Jennings of Paramecium as material for the study of clones, genetically equivalent to pure lines, confirmed Johannsen's analysis for an animal.

The third great development of the first decade, lending exact genetical meaning to Johannsen's theoretical analysis of Galton's ideas on selection in populations, was Nilsson-Ehle's discovery of multiple factor inheritance and its statistical consequences (actually foreshadowed by Mendel). Pure lines, Mendelian recombination and selection for multiple factors made genetics the irreplaceable basis of practical breeding and established breeding as a quantitative science. Furthermore, Nilsson-Ehle's discovery removed the troublesome blended inheritance from the genetic vocabulary by furnishing a simple Mendelian explanation, capable of experimental proof.

Beyond such establishment of the lasting basis of genetics much was accomplished which foreshadowed future developments. Weismann, though anticipating many of the tenets of modern genetics, had made two major mistakes:

he believed all chromosomes to be genetically alike and he assumed that in development determinants were sorted out by unequal divisions. The facts on sex chromosomes, accumulated under Wilson's leadership, showed that chromosomes are genetically diverse; Boveri's brilliant experiments with sea-urchin eggs proved it beyond doubt, and the facts of regeneration and of somatic mutation disposed of Weismann's unequal cell divisions.

The chromosome theory of heredity, in spite of Bateson's and Johannsen's scepticism, became firmly entrenched as a result of the cytologist's detailed work on meiosis, elucidating the synaptic phenomena and even claiming cytological crossing over as a fact of observation. At the borderline of genetics and morphology the successful production of chimeras by Winkler and their cytological analysis, together with Baur's morphological interpretations, opened a fascinating chapter and incidentally led later to the first experimental production of polyploids. The Oenothera work, though at that time mostly beyond the pale of classical genetics, led among other advances to an appraisal of tetraploidy and the discovery of trisomics by Gates.

Many more facts could be mentioned which, only later, were to come to the fore. The first case of multiple allelism was found by Cuénot. Correns and Baur realized the importance of plastid inheritance and anticipated the interest in extranuclear heredity. Cuénot and, afterwards, Baur analyzed cases of lethal factors. The Bateson school (Miss Saunders) began with the chemical study of plant pigments which was to lead to what is now called biochemical genetics, and Garrod did the same for the chemistry of hereditary metabolic diseases of man. As an historical oddity it might be mentioned finally that Lamarckism still remained the credo of paleontologists all over the world, also of many European zoologists and most French biologists. It was the time when most of Kammerer's work was published and when Semon tried to give the doctrine a philosophical underpinning in his theory of the Mneme. Kammerer even tried to ride the wave of Mendelism when he claimed that

his experimentally produced midwife toads which had stopped midwifing could be crossed to normal toads and that midwifing segregated from non-midwifing in a 3:1 ratio if the cross was made in one direction, but 1:3 in the oposite direction. (I am convinced that these crosses were never made.) Those geneticists who at that time still took the Lamarckian claims seriously assumed that parallel induction could sometimes occur; that is, that the same environmental agency may produce a modification and simultaneously a phenotypically identical mutant. We should say today that in the same individual a mutant and phenocopy had been produced. It was even claimed by Tower that he had accomplished this experiment.

After this diversion into the childhood diseases of genetics, we may finish the appraisal of the Gargantuan infancy of our science by stating that after a ten-year struggle for a place in the sun the still small group of geneticists felt that they could proclaim genetics as an established science. The private conferences which Bateson had convoked from time to time were enlarged in 1911 into the first International Conference on Genetics, held in Paris. It was wholly directed and financed by the seed firm of Vilmorin whose clever and energetic head had realized the supreme importance of genetics for plant breeding. Official French biology remained conspicuously absent, and few biologists from other countries attended. Johannsen, Cuénot and the Bateson group were the central figures, Baur and Nilsson-Ehle the oncoming young men. Though international, it was still a meeting of a small group regarded more or less as outsiders by their fellow biologists. However, the first genetic periodical had already been started in Germany, soon followed by one in England. Teaching of genetics had begun in a few universities. The technical book of Bateson on Mendelism and Punnett's semi-popular booklet were followed by Johannsen's great book, and the first two general textbooks of genetics appeared in Germany. Finally, right at the end of the period, Bateson, Saunders and Pun-

nett had discovered a fact, partial linkage, which was to become the focal point of work in the next decade.

You may reproach me with spending so much time upon the childhood of genetics instead of concentrating on the problems of today. The reason is that too many of the younger generation, overwhelmed by the amount of recent work, do not realize the gigantic size of the contribution made by the pioneers in the short space of ten years against serious odds and handicaps. They wrote the alphabet of genetics, without which no one working today could do his spelling. In a golden jubilee talk they deserve the lion's share.

The second decade of genetics is most conspicuous for the initiation of Drosophila genetics under the leadership of Morgan, a *primus inter pares* in a unique team of scholars. Their work ended the period of discussion of the chromosome theory of heredity by proving it beyond doubt. The establishment of the linkage groups paralleling the chromosome sets, and the discovery, by Bridges, of non-disjunction with the complete parallelism of genetic and cytological facts settled this problem forever, though Bateson refused to be convinced to his very end. The study of crossing over and its numerical rules, the brilliant idea of equating crossing-over values with map distances and the almost miraculous consistency of the individual data established the classical theory of the genes as the particulate bearers of the hereditary traits, arranged in linear order on the chromosomes. Simultaneously, gene mutation was shown to be the basic phenomenon of genetics. Among the many special discoveries incidental to this work were the realization, by a number of independent workers, that sex-linked inheritance is the consequence of the location of such mutants in the X-chromosome; the proof that series of multiple alleles were different conditions of one and the same locus; the discovery of mutant loci without special visible effect acting as modifiers of the effect of a main gene, with the consequence that selection of modifiers could explain apparently

non-conforming results of selection for the expression of mutant traits; the better understanding of lethal loci and the discovery of balanced lethals; and finally, the genetic discovery of deficiency and duplication and what turned out to be inversion.

Though this work occupied the center of the stage in the second decade, both by the volume of important new facts and their bearing upon genetical theory, important progress was also made in other lines, much of which came into its own only much later. Genetics established the first experimental contact, as opposed to theoretical discussions, with the doctrine of evolution. This started with the discovery by de Meijere that some of the famous cases of mimetic polymorphism in butterflies had a rather simple Mendelian basis. In trying to explain this in terms of selection, Punnett enlisted the help of the mathematician Hardy and thus initiated the work in population genetics. Another famous case of selection observed in nature, the spreading of so-called industrial melanism in moths, was subjected to Mendelian analysis and considered in the light of the first rudiments of population genetics.

Nilsson-Ehle's discoveries were brought to the fore, especially in plant genetics, when East showed multiple factor inheritance to be typical for most quantitative characters. This work, aided by the already established concept of genetic modifiers, assumed at once a huge importance for plant breeding and animal husbandry and led the quest for the genetic basis of selection beyond the point where Johannsen left off.

It was also in this decade that the modern work on the genetics of sex was begun. The discovery, in a moth, of the phenomenon of intersexuality and its explanation by quantitative relations between the determinants of the two sexes present in either sex, the so-called balance of the sex factors, offered a solution for the problem of sex-determination. It added, also, to the stock of basic genetic concepts the idea of the balanced interplay of gene-controlled reactions (also called, less correctly, genic balance) and thus

made possible the step from static, statistical genetics to dynamic, physiological genetics. Actually, in this decade a small number of geneticists became interested in linking genetics and development. They analyzed facts of development under different genetic conditions, and by comparing the phenotypes in different combinations of genotype and environmental action tried to form ideas on the action of the genes in controlling development. It is probably due to the disruption of communications by the first world war that most of this type of work came to fruition only in the next decade.

Among the many additional lines of progress made in the second decade the following may be said to have contained the seeds of great future development. In the realm of cytogenetics Federley found the surprising features of chromosomal behavior in species hybrids of moths which anticipated much of the later work and revealed the cytological basis of the not yet discovered allotetraploidy. These findings share, with Rosenberg's analysis of the chromosomes in species hybrids of Drosera, the honor of inaugurating the modern epoch of cytogenetics. The work of Correns, followed by Baur and East, on self-sterility started an important line of genetical analysis of problems which had baffled Darwin. The first foundations for the elucidation of the complicated Oenothera problem were laid. Genetical analysis of one type of gynandromorphism, frequent in Drosophila, began to clear up this interesting phenomenon in terms of distribution of sex-chromosomes. Finally, human genetics was made both popular and unpopular by Davenport and studied on a large scale.

If there had taken place another international meeting around 1920, it would have been very different from the semi-private conference of Paris. Genetics had now come into its own completely. It had conquered animal and plant breeding, with some of the practical breeders being among the best fundamental geneticists. There was no longer a textbook of zoology, botany, or general biology nor a lecture course in these fields which did not give a fair

share to the new science. Genetics had become completely integrated with cytology and had also begun to reach into experimental embryology, medicine and anthropology. Professorships and research institutes of genetics had been founded in different countries, France and Russia being the most conspicuous exceptions. A number of special periodicals were published, young biologists flocked to the fascinating new science and only a few diehard biologists were left to criticize and detract.

The third decade saw genetics still progressing at a rapid pace, elaborating old problems and opening new vistas. The mapping of chromosomes by the crossover method continued successfully and was extended to new organisms with results in harmony with the classic case. Different Drosophila species, snapdragons, sweet peas, silk worm and especially maize were in the foreground. The importance of this work was enhanced by coinciding with a new spurt of detailed cytological study of the chromosomes, especially in plants, which infused cytogenetics with new ideas. The most intricate details of meiosis, the role of spindle fiber attachment and the behavior of chiasmata in the meiotic prophase were elucidated and helped in the understanding of special genetic behavior. Polyploidy in nature and in experiment became a major subject of plant cytogenetics. An important discovery was the proof that allotetraploidy, already predicted theoretically and also established as a cytological possibility in the species crosses of moths, could be produced experimentally, first in Nicotiana and then in the famous crosses of cabbage by radish. Soon it could be shown, first in Nicotiana, that allotetraploidy is a major feature in the origin of plant species. Another important discovery was that genetically proven deficiencies, at least in one case, were also cytological deficiencies. Another major group of chromosome rearrangements, translocation or segmental interchange was discovered for cytology when its consequence, ring formation, was correctly interpreted by Belling, a feat which opened a large field of cytogenetic work. The chromosomal mutants of Datura, already dis-

covered in the second decade, could now be completely understood as trisomics, aneuploids of different grade and products of segmental interchange.

One of the most brilliant feats of genetical analysis was the surprising solution of the baffling Oenothera case. De Vries and his followers in different countries had accumulated a body of completely aberrant and incoherent facts in the crosses of races and species of Oenothera. The whole riddle was solved, when, starting in the second decade and finished in the third, it was shown in a brilliant analysis that the Oenotheras are complex-heterozygotes and the different complexes and their interplay were unraveled. The analysis was pushed to its end when, on one hand, the concept of balanced lethals furnished an interpretation of the constant heterozygosity and on the other, when the cytological findings of the translocation chains, involving different numbers of chromosome pairs, provided the visible demonstration of the results of the genetic analysis.

One of the most consequential discoveries of this decade was the experimental proof, based upon an ingenious method of catching all sex-linked lethal mutants, that X-rays produce mutations at a high rate. This was soon followed by the establishment of the rule that the mutation rate is proportional to the dosage of radiation. This opened the field of radiation genetics which first brought physics into the realm of genetics and enabled physicists to analyze the possible happenings when a mutation is induced and to speculate on the type of material which may permit such effects. Other radiations as well as temperature shocks were added soon to the mutagenic agents.

Less spectacular work, but still of great importance for the progress of genetics, was done in a series of other fields of our science, some of which led also to integration with other disciplines. In the preceding decades, the study of plant and animal taxonomy had undergone a considerable revolution. The interest had shifted from the species to the subspecific categories, their distribution, interbreeding or isolation and adaptive qualities. Some geneticists who had

been aware early of the changes in taxonomic outlook, which twenty years later were called the new systematics, set out to explore the subspecific level by combined taxonomic and genetic analysis, the latter under proper exclusion of environmental effects. Such experiments with Peromyscus and Lymantria revealed the genetic basis of subspecific variation and proved their adaptational nature for different habitats in many cases. A similar exploration on the specific level, practically excluded in animals, could be made in the plant Crepis where, in addition, chromosomal differences come into play. On a purely cytological level much information was gathered, both in animals and plants, by the study of chromosomal similarities and differences in groups of species, and ideas were developed as to how these differences may have originated in view of the known cytological rules concerning chromosomal breakage and union, segmental interchange, polysomy and polyploidy.

Evolutionary genetics entered a new period also from a different angle, the beginnings of which go back, as we have already seen, to the second decade. Mathematically-minded geneticists and genetically-minded mathematicians worked out the quantitative consequences of selection of mutants in populations under different conditions of selection and mutation pressure and different systems of breeding. They established the rules which apply to the survival and spread of mutants and their combinations in interbreeding or isolated populations of different size. Strict Darwinism had subsided in the early period of Mendelism because sudden mutation seemed to be in contradiction with Darwin's idea of slow transformation by selection in a continuous chain of variation. Now it was reintroduced in an extreme form as Neodarwinism, gaining additional influence upon other fields in which population studies were indicated, as human heredity and animal husbandry.

Actually the specific features of human genetics had already made the use of mathematical statistics imperative in pre-Mendelian times as the names Quetelet, Galton

and Pearson signify. A mathematically-minded physician, Weinberg, in the first decade of Mendelism, had realized the need for special mathematical methods in the study of human heredity as well as the importance of population studies in the same field, and continued his classic contributions to the time under discussion. This made it possible, for example, in one major field of human genetics, the blood groups, for a mathematician to devise a correct Mendelian explanation via population mathematics. Altogether, human genetics made great strides in this decade, partly prodded by the wild antics of the eugenists, partly in the wake of the introduction of new methods, foremost among them the study of identical twins.

Among the many lines of genetical work started in the second decade and continuing their growth in the third should be mentioned the study of sex-determination. When triploid intersexuality, which Standfuss had discovered and correctly interpreted in moths, was found also in Drosophila it became possible to envisage the theory of the balance of sex determiners in terms of chromosome numbers. Though this is possible only in triploid intersexuality, not in the more frequent diploid one, it opened new methods of detailed attack on the chromosomal level. New objects for the study of genetics of sex were introduced which had their own peculiarities. Among other features, attention was again drawn to the Y chromosomes, the genetics of which had just been studied in Drosophila and which, in such animals as fishes—much later also in plants—entered the study of sex determination. In this field a new contact with a neighboring science was established when the respective role of genetic determiners and hormones in the control of sex was studied, especially in amphibia, and the problem of genetic *vs.* phenotypic sex determination was posed in the important studies with Bonellia and simple algae. For the first time also sex control in monoecious plants could be understood genetically in the remarkable work on sexual abnormalities in maize.

Only a word should be said about physiological genetics

in this period which continued quietly to accumulate material for the analysis of genic action in the control of development. Mutants of Drosophila, mice, fowl, guinea pig, flour moth, fresh-water shrimp and Lymantria, were studied in different genetic conditions as multiple alleles, comparing by observation and experiment the respective processes of development in different genotypes. The problem of dominance was attacked and a new tool was introduced in the dosage experiments made possible by the elaborate Drosophila technique, though their principle had been laid down in the early time of Mendelism in the study of endosperm mutants and later, more elaborately, in the work on intersexuality. The discovery of phenocopies opened another avenue of approach to problems of physiological genetics. The third decade saw also some geneticists probing further into the dubious role of the cytoplasm and its inclusions in plants, the study of which was most successfully continued in Oenothera. Indications were found, in two different animals, Lymantria and Habrobracon, that the action of the same gene may be different if taking place in different cytoplasm. The most extensive work was done with mosses and Epilobium, where a number of morphological characters seemed to be controlled in the main by the cytoplasm so that the existence of a "plasmon" different from the genome was assumed by Wettstein, though subsequent work was not completely confirmatory. Another tentative approach was made in infusoria where an after-effect of specific treatment lasting over generations, was described by Jollos as "Dauer-modification." Much of this work on the cytoplasm was to affect genetic thought only much later.

Many more special lines of progress might be indicated, such as the work on inbreeding, heterosis and self-sterility in plants, on gynandromorphism in animals. Suffice it to conclude the review of the third decade by stating that new objects of genetical research were introduced which enlarged considerably the latitude of our knowledge and served as examples of the different ways by which nature can accom-

plish its goal, ways the origin of which it is not easy sometimes to explain. I mean such work as that on the genetics and cytogenetics of Sciara and Habrobracon. After many trials the numerous oddities of the chromosomal cycle in Sciara were brought into line and the aberrant genetical behavior interpreted. Habrobracon furnished a wealth of information on known genetical features as well as on aberrant and interesting behavior, among them a new type of sex determination.

Thus, after three decades the science of heredity had reached a monumental size and had established connections with many other disciplines which gained new insight into their own problems from such contact.

The fourth decade was to broaden, as well as to deepen, the different channels of our science and in addition to plan and to build broad stretches in new directions. One may say that the study of crossing over as a tool of analyzing the material basis of heredity was brought to an end by the addition of the visible proof of chromosomal exchange when, in maize and Drosophila, the simultaneous use of genetic and structural markers made visible the actual result of crossing over.

Meanwhile cytogenetics had made or was to make two of its greatest advances. The detailed structure of maize chromosomes was so completely worked out that it became possible to relate many genetical facts to chromosomal structure. This was followed by the discovery of the most powerful cytogenetic tool. Drosophila, known as a poor material for cytogenetic study, became the foremost object by the discovery of the meaning of the structure of the salivary gland chromosomes and the application of their study to problems of genetics. Deficiencies, inversions, duplications and translocations could be really seen and exactly defined. The genetic analysis of the seriation of mutant loci could be translated, for many points, into visible localization, in complete agreement with both avenues of approach. Chromosomal rearrangements could be studied quantitatively, and it could be demonstrated that X-rays produced chromo-

some breaks in principle according to the laws established for mutants. Though some large plant chromosomes allow the establishment of the occurrence of breaks by their direct or indirect consequences, only the salivary chromosomes make it possible to deal with such minute sections of a chromosome as would be invisible in ordinary chromosomes and, even, to make them subject to biochemical study. Of the many important consequences of these discoveries only one may be mentioned which started a big wave of work. I mean the detailed comparison of the cytogenetics of different Drosophila species. It led not only to important discoveries on the relative gross architecture of the different chromosome sets as well as of detailed cytogenetic parallelisms between nearly related species, but also suggested an intricate study of taxonomy, cytology, distribution, ecology, inter-sterility of the Drosophila species which made this formerly insignificant genus of flies the best known of all genera of animals.

Less dramatic but full of meaning for future work were other cytogenetic studies. I mention the discovery of heterochromatin and its role in the architecture of the chromosome and the elucidation of the relation between chromosomes and nucleolus. A powerful weapon was added to the cytogenetic armory with the discovery of the colchicine technique which permitted the production of auto- and allo-polyploids where required for analytical or practical purposes. The introduction of monosomics of Nicotiana as an ingenious tool of cytogenetic analysis constituted another remarkable advance.

While these discoveries conquered new provinces for cytogenetics and general genetics, some of the older fields started out in new directions. In passing it might be said that applied genetics took advantage in this and the foregoing decade of all new discoveries and put them to practical use. Genetics thus remained the beacon of progressive, scientific plant and animal breeding. Mutation, factorial recombination, selection for multiple factors and modifiers and their transfer by outbreeding, inbreeding and heterosis,

the mathematical appraisal of systems of breeding and also polyploidy are some of the genetical methods which led to success. They made plant breeding and animal husbandry one of the brilliant demonstrations of the beneficial impact of fundamental research upon progress in man's control of nature.

In pure genetics the great success of the work on induced mutation created a new interest in spontaneous mutation, the cause of which remained unknown in spite of many guesses. Many remarkable facts were discovered, none of them completely understood, which may lead one day to better insight. There were found to be lines of different hereditary mutability and in some cases definite sections of a chromosome could be shown to affect it, though in other cases a gross feature of the chromosomes, stickiness, seemed to be responsible. A number of cases of an explosive mutability were encountered, but no real analysis could as yet be performed. Facts became known in such different organisms as yeasts and Drosophila, pointing to the possibility that mutation is not completely haphazard and may even be directed. Finally, the analysis of many cases in plants of so-called mutable loci presented new aspects of the mutation problem which are not yet sufficiently understood though full of hidden meaning.

Especially large were the strides which physiological genetics made in this decade. Much new work was done in relating genetics with development, using different methods of environmental action and embryological experimentation in connection with genetic diversity. In addition to work with Drosophila and rodents, important discoveries were made in the analysis of the wing pattern of the flour moth. All of this was, however, a continuation of former work, using more or less the same method of attack. A completely new province was conquered, when, in the flour moth and in Drosophila, the method of larval transplantation of anlagen was introduced which made it possible to test for the presence of gene-controlled substances needed for production of normal or mutant eye pigment. It was

first thought that these ingenious experiments revealed the production of active agents or hormones by the respective genes. But it turned out that chemically identifiable pigment precursors were involved. Thus a type of genic control of individual steps in the synthesis of complicated compounds was established. In a general way the results concurred with similar work on chemical genetics of flower colors in plants as well as with earlier, but largely overlooked, work on the biochemical genetics of metabolic disturbances in man. Actually this new work meant the exact biochemical analysis of the genetic control of pigmentation involving the interplay of chromogens and oxydases which have been tried in a more generalized way since the early days of Mendelism for the pigment of rodents.

Thus we come to the last decade of genetical research, the end of which we are celebrating. Up to this point we tried to enumerate and evaluate objectively, though hardly in a completely detached way, the triumphant progress of genetics. With the last decade we enter a period, the accomplishments of which are clearly present to all of you who yourselves have been busy writing this record. Moreover, the following symposia will review the technical details of such work as has been foremost in your minds during these years. Thus I propose to present only a bird's-eye view of what I consider the most important trends. I may be excused if I enliven occasionally the necessarily superficial review by a more subjective approach. This will sometimes not appear in accord with the views held by many of you. I beg to state that dissenting interpretation in no way lessens my respect and admiration for the work which I am inclined to interpret in my own way.

One of the most outstanding events of the last decade is, of course, the mushroom-like growth of the genetics of lower organisms. Already in the childhood of genetics, Hansen, Blakeslee, Kniep and Jennings had laid the foundations of such work. A little later the Hartmann school studied protozoa and the genetics of sexuality in algae. It was the discovery by Jennings of the plus and minus strains

in infusoria which made this group of protozoa a major object for genetical research. The success of the work with the life cycle of the Ascomycetes suggested this group as a useful material for genetical study. All this induced the bacteriologists to consider their work in the light of modern genetics and geneticists joined them in laying the foundations of bacterial genetics. Most amazingly, though foreshadowed by an early but hardly correct comparison between bacteriophage and gene, virus became a remarkable object of genetic investigation.

Looking at all this from a bird's-eye view, as proposed, the first fascinating line of work in the field of the lower organisms is the study of what has been termed biochemical genetics. We described before the earlier endeavors to link mutant loci with definite steps in biochemical synthesis of an end product which had led to such concepts as considering the gene as an enzyme which catalyzes reactions producing an enzyme, hormone, formative stuff for the final steps of embryonic determination. The development of ingenious methods for the study of mutant effects upon the synthesis of amino acids and vitamins in Neurospora went far beyond such general statements and produced an immense body of facts. It can already be said that this work has conquered an important new land in the realm of biochemistry with still greater prospects ahead. On the genetical side it seemed to lead to a broad generalization. When it was shown that mutants producing a biochemical deficiency prevented one single step in the ladder of synthesis of such stuffs, it was concluded that the mutant condition prevented the formation of the specific enzyme controlling the respective step. This again was interpreted to mean that the normal gene is responsible for the production of this enzyme. Therefore, a one–one relationship between genes and specific enzymes was considered to be demonstrated. Though there can be no doubt that such a relation exists in some cases, the sweeping generalization did not seem quite justifiable. The facts brought to light since in

the same line of work have poured some water into the wine of theory, and it is possible that biochemical geneticists share now the scepticism of some geneticists outside this field as to the validity of the generalization.

A comparable body of results linking genetic sex-determination in the unicellular green flagellate Chlamydomonas with very specific quantitative production of definable substances has been claimed. If the facts are confirmed—some of which still look too perfect to be true—the most elaborate details in the chain: gene—biochemical product—control of a general organismic feature (sex in this case) will have been brought to light.

Similar fascination attaches to the genetic work with infusoria which has revealed a large body of facts on mutation, Dauer-modification, sex and nuclear function in these forms. The most discussed and interesting part of this work deals with the interrelation of nuclear-genic material and the cytoplasm. It seemed that here, finally, the existence of plasmagenes could be proved, a concept which had cropped up for a long time, especially in plant genetics. Actually some geneticists talk of plasmagenes as if they were a fully established fact. The last developments of the brilliant work on the killer character in Paramecium have, I think—probably with many others—removed for the time being the most important factual basis from under the plasmagene concept.

We may pass over the genetics of yeast as the interesting facts discovered in this field seem to fall in line with general genetic concepts as may be expected in a clearly cellular organism. Only a few years ago such expectations would not have been entertained for bacteria. The genetical study of this class has indeed brought forth many unexpected discoveries. The most surprising fact is that in a general way genetics of bacteria is similar to that of other organisms. Though the proof that bacteria have an organized nucleus and even chromosome-like structures seems now around the corner, no cytological facts have been established which would prove beyond doubt the existence of a material basis

for Mendelian genetics, mitosis, meiosis and a sexual cycle. But genetical facts imply that all this must exist. By the use of specific methods which circumvent the difficulties created by the peculiar features of bacterial culture it has been proven that factorial mutation exists and that it can be speeded up from the low incidence, comparable to that of higher organisms, by the action of X-rays and mustard gas. If strains, with at least two different mutants each, are combined they behave as in a Mendelian cross and recombination occurs. Even linkage and crossing over seem to be observed. This requires of course the proper cytological basis which at present must be postulated to exist. There are quite a number of specific features of mutation and selection which parallel known genetic facts. Only one result, as far as I can see, is really baffling to the geneticist and has led to many speculations. This is the proof for pneumococci and Escherichia that a specific desoxyribonucleic acid extracted from one typical genetic form and added to the medium of another genetically controlled strain of different phenotype makes this latter strain transform hereditarily into the phenotype from which the nucleic acid was first extracted, even after the complete removal of that nucleic acid from the environment. The inference has been made that here a genic product of one form has entered not only the body of another one but has been incorporated somehow into its genic material with its original function. I wonder whether it should not be worth while to explore these facts from the standpoint of the genetic concept of norm of reaction. It is known that certain amphibious plants produce completely different leaves when growing in air or in water. This is due to their genetically controlled alternative norm of reaction. If different cytoplasmic constitutions of a bacterium may act as different environments for a hereditary alternative norm of reaction, the incorporation of the foreign nucleic acid into the cytoplasm may result in the changed action of the genic material without any change of the gene itself.

While it looks, at least at this moment, as if bacterial

genetics were not different in principle from the aspects and laws of genetics in other organisms, the newly developed field of virus genetics offers some really exciting facts. Mutation has been proven to exist, and in the simultaneous presence of different mutants of bacteriophage the possible recombinations are recovered in the population, and the very remarkable details regarding the sequence of events after the entrance of a phage into its host have led to interesting considerations. The conclusion seems unavoidable that the recombination is the consequence of a break-up of the phage into primary genetic particles which may recombine again into the phage. Additional hypotheses on the methods of reassemblage of the units would take care of linkage and crossing over, if such were found. I trust that we shall hear more of this these days. The important point for genetics as a whole is that hope has been raised that here an archaic condition of the genetic mechanism may be explored which will one day throw real light on the biochemical basis of heredity.

There are a few more recent discoveries which may lead in this direction which I beg only to enumerate without further discussion. I mean the discovery of chemical mutagenic agents, further the discovery of chemical induction of specific phenocopies and the still rather enigmatic work on the role of a kind of cytoplasmic constituent in the causation of CO_2 sensitivity in Drosophila.

All this now leads to the central problem. As long as genetics has existed, the ultimate problem has been the nature of the gene, its reduplication and mutation. At each step in the development of genetics more or less specific ideas were formed such as considering the gene to be an autocatalyst, or a group of molecules of definite size, or a single molecule, or a side chain in a supermolecule. In a general way the gene was considered as a definite particle, different and independent of the next one like a series of beads on a string. An increasing number of geneticists are no longer content with this, the classical concept. Among the facts which have led to such questioning, the existence

of position effect is the most prominent one. This means, in terms not prejudicing the explanation and overlooking some special types, that a chromosomal rearrangement with a break near a definite locus acts as if the locus had mutated in its typical way. All mutants and all position effects in such a section of a chromosome behave as multiple alleles. The analysis of such facts has led an increasing number of geneticists to doubt the existence of a particulate gene molecule which mutates by an intramolecular change and to replace the classical theory by a more flexible one involving different sections of the chromosome up to the entire chromosome as possible units of action. The fate of such ideas, as well as of those which they are trying to replace, depends largely on the progress of protein chemistry, the chemistry of nucleic acids in relation to protein synthesis and the chemistry of supermolecules and of viruses. Work done in recent years in introducing immuno-chemistry into genetics will probably share in solving the ultimate problem of genetics and has already furnished some interesting ideas on the visualization of the basic process of genetics, genic reduplication.

A third field of genetics which is progressing most conspicuously in our time is evolutionary genetics, especially population genetics. Built upon such early discoveries as the Hardy-Weinberg law, the mathematical study of selection and mutation pressure, the concepts of preadaptation, the ecotype and the ecological niche, further the study of quantitative inheritance and selection of modifiers, and finally the changes in taxonomic outlook led to a reawakening of Darwinism, actually a hyper-Darwinism. A large body of experimental work was and is being done in genetically mixed populations under the impact of adaptational selection or as a consequence of genetic drift in restricted groups of a population. Problems of isolation, interfertility and sterility, migration, geological changes of habitat enter the picture as well as genetic mechanisms for the preservation of adapted combinations. The large material already accumulated reveals clearly the workings of selection, adapta-

tion and drift in interbreeding populations with genetic heterozygosity. It has led to a Neo-Darwinian doctrine which has been adopted also by many paleontologists. The great and admirable enthusiasm of many workers in this field has led them to the belief that they have established the exact foundation for the Darwinian concept of evolution by accumulation of minute mutations by selection or drift. But the number of those biologists is increasing who ask themselves whether it is really possible that the small hereditary variations by which adaptation to the general existential features of the environment is accomplished can be integrated into the major evolutionary changes which produce the perfect adaptations of the entire organism in the huge diversity of types. They also ask themselves whether chance combinations of such small variants, if accumulated by genetic drift, can ever produce those adaptations, say the morphology and physiology of whales or hummingbirds, the mechanism of action of the woodpecker's tongue, or the snake's poisonous fangs and a thousand others.

We come to the end of this admittedly incomplete record of the growth of genetics in the first half century of its existence. Looking back at the history of thought we realize that from time to time discoveries are made and ideas proposed which, though starting in an apparently limited field, at once illuminate all fields of human knowledge and force them to adapt their mode of thinking to the new insight. In the modern history of science such an event was Galileo's experimental work which started the new era of inductive science. Another one was the establishment of the theory of evolution by Darwin. Accepting the risk of being reproached with carrying too far the spirit of a jubilee, I state that the rise of genetics is another instance of discovery of new facts and of the conception of new ideas which have deeply influenced many fields of intellectual endeavor and the end is not yet in sight. Taxonomy and comparative anatomy, embryology and cytology, physiology and ecology have felt the impact. Evolutionary thought

has been rejuvenated; paleontology has been forced to reconsider its basic tenets; zoology and botany have been welded together; eugenics and anthropology were given a solid basis; psychology and sociology have begun to take notice; biochemistry has not only been assimilated as a tool, but has received important stimulus in return. Medicine has been deeply influenced; bacteriology owes to genetics a new upsurge; and virology has assumed added importance; the philosophy of the organism is reconsidered and theoretical physics begins to introduce genetics into its most subtle deliberations. Geneticists may take reasonable pride in such performance and still more in the fact that progress has not slackened its pace and that the future holds no limits to further conquests.

More than four hundred years ago one of the most colorful figures of the Age of Reformation, Ulrich von Hutten, overwhelmed by the happenings of his day and the hope of a still greater future, exclaimed the famous words, "*Es ist eine Lust zu leben.*" ("It is a joy to be alive.") May I adopt this exclamation for the status of genetics today and close with the words: Geneticists of 1950, *Es ist eine Lust zu leben!*

X-RAY INDUCED GENETIC EFFECTS IN GERMINAL AND SOMATIC TISSUE OF *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION

THE mechanism by which a gene changes into a new allele remains unknown, even though much genetic research has been directly or indirectly motivated by the hope of providing some insight into the gene mutation process. A favorite tool for attacking the mutation problem has been the use of radiations (and more recently other mutagenic agents) in order to stimulate the production of mutations. From such investigations some knowledge has been gained of the conditions under which radiation-induced mutations are produced. It has been amply verified that the number of mutations produced by X-rays is linearly proportional to the total dose applied, even when the total dose received is very small (see Spencer and Stern, 1948). Further, the number of mutations produced is independent of the rate of dosage (Uphoff and Stern, 1949), and is likewise independent, over a wide range, of the wave-length of the radiation employed (Fricke and Demerec, 1937). These facts led Timoféeff-Ressovsky, Zimmer, and Delbrück (1935) to a theoretical explanation of radiation-induced mutation on the basis of the target theory. This concept of direct radiation action on the genetic molecule has been especially championed by Lea (1947), and extended to the breakage of chromosomes and to the inactivation of viruses and bacteria as well.

More recently another interpretation of radiation-induced mutation has developed, based in part on the effects of such

[1] The data in this paper have been taken from a thesis submitted to the Graduate Faculty of the University of Missouri in partial fulfillment of the requirements for the degree of doctor of philosophy.

external factors as oxygen tension and temperature on the rate of induced mutation. King (1947), Baker and Sgourakis (1950), Riley and Giles (1950), and Fabergé (1950) have presented evidence indicating that radiation-induced mutation is produced through an indirect action, in which the primary effect is the formation of active radicals in the aqueous medium surrounding the gene. By chemical reactions with these radicals, or more likely with H_2O_2, the gene is secondarily modified. The work of Haas *et al.* (1950) and Wagner *et al.* (1950) on the production of mutations in bacteria and *Neurospora* by irradiation of the medium into which the organisms are subsequently placed supports such an interpretation of indirect radiation action.

However, it has long been established by Muller and Mott-Smith (1930) that the incidence of spontaneous mutation can not be accounted for by the amount of ionizing radiation impinging on organisms in nature. Thus, it may be questioned if the process of radiation-induced mutation, whether it be by a direct or indirect action, or both, is identical with the spontaneous mutation process. In this connection an interesting paradox exists in the literature of radiation genetics. On two counts *Drosophila* workers have claimed that the induced and spontaneous mutation processes must be qualitatively similar. First, radiation can produce reverse mutation of recessive mutants. As Patterson and Muller (1930) first showed, X-rays can cause reversions of X-ray induced recessive mutations. Timoféeff-Ressovsky (1933, 1939) also succeeded in reversing X-ray induced mutations by further irradiation. Johnston and Winchester (1934) produced several reverse mutations in a large-scale experiment. Few other positive reports have been made, except for reversals of position effects. Secondly, it is common experience with *Drosophila* that among X-ray induced mutations, intermediate alleles are not infrequently produced as well as the basic alleles. These two evidences have led *Drosophila* geneticists to conclude that an induced mutation can not be a loss, but is essentially similar to a spontaneous mutation.

In contradiction to these results with *Drosophila,* Stadler and his colleagues, working with maize, have concluded that ionizing radiation produces only gene loss rather than mutation. Stadler and Roman (1943, 1948) compared spontaneous, X-ray induced, and ultraviolet-induced mutations at the *A* locus. They found that all X-ray mutants show markedly reduced transmission through the male gametophyte in contrast to the normal behavior of spontaneous and ultraviolet-induced mutants. Moreover, Stadler (1944) attempted to reverse recessive *a* mutants (of spontaneous origin) by X-rays and found no evidence of induced reverse mutations in a large sample, even though the *a* allele used readily reverted under the influence of the gene Dotted. In maize it appears that ionizing radiation is essentially destructive, and induced mutations are not equivalent to spontaneous or ultraviolet-induced mutations.

Recently, studies with *Neurospora* of the reversibility of biochemical mutants have been carried out by Giles and Lederberg (1948) and Kölmark and Westergaard (1949). These workers reported the successful induction of reverse mutations by X-rays, ultraviolet, and mustard gas. Genetic tests indicated that such reversions are true gene mutations. This result stands in contradiction to that with maize, but supports the original *Drosophila* work. It is not clear, however, whether any of the *Neurospora* mutants treated were of X-ray induced origin.

Because of the many universal characteristics of genes, chromosomes, and the laws of inheritance, it has been tacitly assumed that genes are similar in nature and that the mutation process is basically identical in all living organisms. No proof exists that this is the case, but it would appear strange that in maize true gene mutations can not be induced by X-rays, while in *Drosophila* and *Neurospora* perfectly good mutations result from the action of ionizing radiation. This paradox could be resolved if: (a) genes in different organisms are not fundamentally similar, (b) species differences affect the responsiveness of similar genes to ionizing radiation, or (c) the interpretation of the data is

not reliable, and the mutagenic effect of X-rays is truly the same in maize, *Drosophila,* and *Neurospora.*

Some attempts have been made to repeat the induction of reverse mutations in *Drosophila.* Kaufmann (1942) and Demerec (1938 and personal communication) have accumulated a considerable amount of negative data. Because the question of the induction of reverse mutations with ionizing radiation is important to the evaluation of the consequences of the exposure of human genes to such radiations, further investigation of the capacity of X-rays to produce reverse mutations is desirable. In the present study two aspects of the problem of induced mutation were investigated. First, attempts were made to induce reverse mutation of several recessive sex-linked loci by X-rays in both germinal and somatic tissue of *Drosophila melanogaster.* No positive results were obtained. Second, the X-ray induced direct mutation rate at the w^+ locus was studied in both germinal and somatic tissue. Very similar values were found for the incidence of the mutation of w^+ to w in the two tissues. In contrast, it is suggestive that the spontaneous mutation rate of a given gene, especially as evidenced by unstable genes (Demerec, 1931, 1935, and 1941), is frequently sensitive to the influence of its cellular environment. The possibility must be given serious consideration that the X-ray induced mutation process in *Drosophila,* as in maize, is not qualitatively similar to the spontaneous mutation process.

Material and Methods

Germinal Mutation Studies. Two series of experiments were carried out in the investigation of X-ray induced mutation rates in *Drosophila melanogaster.* Reverse germinal mutation was studied by irradiating males possessing various sex-linked recessive genes and mating them to unirradiated recessive females or to attached-X females. Direct mutation studies were also undertaken, in part to have a check on the efficiency of the irradiation procedure followed, but primarily to obtain data for a comparison with induced somatic mutation rates. In the direct mutation studies

Canton-S wild-type males were exposed and then mated to unirradiated recessive females or to attached-X females.

Reverse mutation data were obtained principally for the yellow (*y*), white (*w*), singed-3 (sn^3), and forked (*f*) loci; a few additional data were also obtained for the vermilion (*v*) and miniature (*m*) loci. Six different stocks were used to provide males for irradiation. They were: (1) $y\ w\ sn^3$, (2) $y\ f^{257\text{-}4}$, (3) $y^2\ w^a\ m\ f$, (4) $y^2\ w^a\ v\ f$, (5) $w^{258\text{-}52}$, and (6) $sd\ f^e$. In the last stock the wing character scalloped (*sd*) was not considered.

At the time of irradiation appropriate males, one to three days old, were placed in gelatin capsules which were supported five inches from the target of a universal type Coolidge X-ray tube. The X-ray machine was operated at 160 volts primary and 4 milliamperes tube current. (Secondary voltage was not measurable.) With a one-mm. aluminum filter the flies received approximately 250 r per minute, as determined by a Victoreen dosimeter. Most of the exposures lasted for 20 minutes, giving a total dose of 5000 r. However, some 17-minute exposures were made, during which the flies received only 4250 r. All exposures were made at room temperature.

After irradiation the males were placed with appropriate unexposed females in quarter-pint milk bottles containing standard cornmeal-molasses-agar food medium, and incubated at 25° C. for three days. At that time all parents were subcultured in fresh bottles for another three-day period, whereupon the parents were discarded. This procedure assured that only spermatozoa which were mature at the time of irradiation were tested for the presence of induced mutations.

Emerging offspring of both sexes were counted and inspected at a magnification of 18 × for the presence of any visible mutations. Counts were discontinued six days after the first offspring emerged unless, as usually happened, the cultures ceased producing earlier. Since only sex-linked loci

were studied, induced mutations were observable in only one sex; the other sex serving as unirradiated controls. Thus, separate control experiments were unnecessary.

Particular precautions were taken in order to eliminate the possibility of contamination being the source of spurious reverse mutations. No stocks were kept in the laboratory which possessed all but one of the same genes present in the stocks used for irradiation. It might appear that wild-type flies would violate this precaution in the case of the $w^{258\text{-}52}$ stock; similarly yellow or forked flies in the case of the $y\,f^{257\text{-}4}$ stock. However, both $w^{258\text{-}52}$ and $f^{257\text{-}4}$ are associated with euchromatic inversions which served as additional markers.

Somatic Mutation Studies. X-ray induced direct and reverse somatic mutation rates were studied only at the white locus. In the direct mutation experiments Canton-S wild-type males were used exclusively. However, reverse mutation studies were carried out in three different white-eyed stocks in which the white alleles were: (1) spontaneous in origin, (2) induced by X-rays, and (3) induced by sulfur-mustard gas. Some additional data were obtained with an apricot stock.

In all the somatic mutation studies larvae of known age were irradiated, and after development at 25° C. the emerging adults were inspected at a magnification of 27 × for the presence of eye color mosaics. Exposures were made 26, 48, and 70 hours after egg laying, and in any one exposure the age of the larvae was known within ± 1 hour. Lower X-ray doses were utilized than in the germinal mutation studies because of the high larval mortality which results with doses much greater than 1500 r. All exposures were made at a total dose of 1250 r or 1300 r. Dose rates of 59, 24, and 15 r per minute were employed.

An experienced observer can usually detect even one-facet eye color mosaics at a magnification of 27 ×, but some small mosaics undoubtedly escape observation. Detection of mosaics can be greatly facilitated by immersing the adults in clear paraffin oil. Not only do mosaics stand out

with startling clarity under oil, but even small pigmentless mosaics can be readily differentiated from those possessing some pigment. None the less, a distinctly subjective factor exists in the detection and classification of eye color mosaics, and considerable experience is essential if reliable data are to be obtained.

The size of all mosaics detected was determined by counting the number of facets included in the mutant area. For small mosaics the count was quite accurate. However, for mosaics containing more than 15 or 20 facets the number of facets was estimated rather than counted exactly. Test counts of some larger mosaics served as the basis for these estimations. Since the majority of mosaics found were relatively small, the recorded facet counts were reasonably accurate.

Experimental Data

Direct Germinal Mutation. Direct germinal mutation studies were undertaken as a control for the reverse mutation studies, and to provide data for the comparison of the mutation of w^+ to w in germinal and somatic tissue. A considerable body of data regarding mutability at the white locus is available from the studies of Timoféeff-Ressovsky (1932 and 1933), Muller (1940), and Bonnier and Lüning (1949). Thus, in the present study only relatively small-scale experiments were carried out, which were sufficient to insure that the stocks used were comparable in their radiation sensitivity, especially at the white locus, to those employed by the other investigators.

Direct mutation was followed for only the *y, w, sn, v, m,* and *f* loci, since these were the loci tested for reverse mutation. Following exposure to 4250 r, a total of 43,849 loci were tested for induced direct mutation, and nine separate viable mutants were detected. For the w^+ locus alone, two viable white mutants were found among 8,997 loci tested for direct mutation. In addition, however, three different individuals were observed whose eyes were unmistakably mutant; but when they were bred, no evidence of change in

the irradiated w^+ locus was found. Apparently, these three flies possessed fractional mutations in which the anterior part of the body was mutant, but not the posterior region. No evidence of gynandromorphism was seen in any of these flies, and all three were fertile. If these cases may be accepted as fractional mutations, mutability at the w^+ locus in the stocks used in the present study was not significantly different from that found by other investigators. The overall mutability of the six loci studied following exposure to 4250 r is about that to be expected on the basis of the various direct mutation constants given by Timoféeff-Ressovsky and Delbrück (1936). Combining all the available data regarding X-ray induced direct mutation of w^+ to w (not to any allele of white), an average mutation constant of about 12×10^{-8} may be calculated. This mutation constant expresses the probability that w^+ will mutate to w following an exposure to one r-unit of X-rays.

Reverse Germinal Mutation. Altogether 166,234 recessive X-chromosome loci, including *y*, *w*, sn^3, *v*, *m*, and *f*, were tested for reverse mutation following exposure to 5000 r. Completely negative results were obtained. In Table 1 the actual data are presented, and for comparison the combined results of Timoféeff-Ressovsky (1939) and Johnston and Winchester (1934) are also shown. It should be noted that both Timoféeff-Ressovsky (1939) and Fryer and Gowen (1942) have incorrectly reproduced the data of Johnston and Winchester, showing more reverse mutations than were actually recorded in the latter's original paper.

Direct Somatic Mutation. Wild-type Canton-S males were irradiated during the larval period, and the developing adults subsequently examined for the presence of white or light eye color mosaics. Three series of exposures were made: 26, 48 and 70 hours after egg laying. In the latter two experiments all adults were immersed in clear paraffin oil during the examination for mosaics, but in the 26-hour series this procedure was not followed. Under oil the difference between pigmentless and colored mosaics is quite evident, and the separation into the two classes is reliable

TABLE I

Summary of Reverse Germinal Mutation at the *y*, *w*, *sn*^{3}, *v*, *m*, and *f* loci, together with the Published Data of Timofeeff-Ressovsky (1939) and Johnston and Winchester (1934). All data have Been Adjusted to 5000 r, on the Basis of a Direct Proportionality of Mutation to Total Dose

Locus	Present study		Published data	
	Number tested	Reverse mutations	Number tested	Reverse mutations
y	49,523*	0	77,835*	1
w	49,775	0	216,256	8
sn^3	43,856	0	...	...
v	1,084	0	78,279	3
m	2,374	0	50,575	3
f	19,622	0	138,180	19
Totals	166,234	0	561,125	34
Controls	208,634	0	152,352†	0

* Number tested = actual number examined x $\frac{\text{actual dose}}{5000}$.

† Johnston and Winchester reported no control data.

for all except single facet mosaics. Although one-facet mosaics may be readily detected under oil, their separation into colorless and colored classes is usually uncertain. However, only in the 70-hour series were one-facet mosaics encountered in any appreciable frequency. In Table 2 the results of the three series of exposures are recorded, and in Fig. 1 the size distribution is plotted for all the mosaics, both white and pigmented, which were found in the 48- and 70-hour series.

The white mosaics are considered to result from somatic mutations of w^+ to w in cells of the eye anlagen, since no other locus affecting eye color is known to eliminate pigment entirely. On the other hand, mosaics containing some pigment may represent mutations to intermediate alleles of white; or mutations of other sex-linked eye color loci such as ruby, garnet, etc.; or dominant autosomal mutations. No method exists to distinguish between these different possibilities. However, the pigmentless class of mosaics

TABLE 2

Incidence of White and Light Eye Color Mosaics Following Irradiation of + Male Larvae 26, 48, and 70 Hours after Egg Laying. At 26 Hours 1250 r Was Given. At 48 and 70 Hours 1300 r Was Given.

Age at exposure	Rate of dosage r/min.	No. ♂♂ examined (a)	Number of mosaics			Av. no. facets (c)	Per cent. Mosaics/eye	Somatic mutation constant
			Total	White (b)	Colored			
26 ± 3 hr	59	728	12			27	0.8	11.3†
26 ± 3	24	1025	15	(19)*	(18)*	38	0.7	×
26 ± 3	15	929	10			28	0.6	10^{-8}
47 ± 2	97	113	7	5	2	8	3.1	14.2
48 ± 3	59	1013	75	36	39	7	3.7	×
48 ± 3	24	145	14	6	8	7	4.8	10^{-8}
70 ± 2.5	59	776	46	26	20	5	3.0	6.5×10^{-8}
Controls		2719	1	1	0	6	0.02	...

* The 37 mosaics of the 26-hour series have been arbitrarily divided into white and colored classes in the same proportion as found in the other series in which the examination of the adults was made under paraffin oil.

† Mutation constant $= \frac{(b)}{2(a) \times \frac{750}{(c)} \times \text{dose in r}}$, assuming the adult ♂ eye has 750 facets.

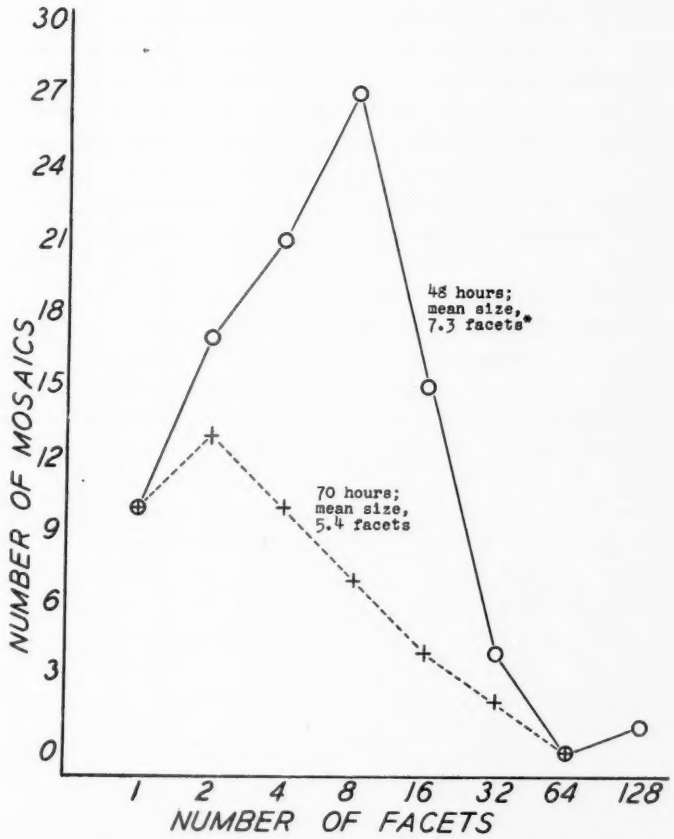

FIG. 1. The size distribution of all mosaics resulting from the irradiation of + male larvae 48 and 70 hours after egg laying. Examination of adults was made under paraffin oil.

* The mean size was calculated neglecting the single large mosaic.

provides a basis for computing a somatic mutation constant for the particular mutation step of w^+ to w. If, in addition to the incidence of white mosaics produced by a given X-ray dose, the total number of w^+ loci irradiated can be computed, then the somatic mutation constant may be calculated by dividing the number of white mosaics found

by the number of w^+ loci irradiated multiplied by the dose in r. Assuming that each primordial eye anlage cell contributes equally, on the average, to adult eye tissue, then the fraction of the eye occupied by a white mosaic reflects directly the number of w^+ loci present in the eye anlage at the time of irradiation. For example, if four w^+ loci are present in an eye disk when the larva was exposed and a somatic mutation at one w^+ locus occurred, then one-fourth of the adult eye should be white. However, only if the mosaics produced comprise a relatively homogeneous group and show a random size distribution as well as a random distribution throughout the eye will the estimate of the number of loci irradiated be reasonably accurate. When exposures are made too early or too late in development or when the age spread of the larvae exposed is too great, then invalid estimates are likely to result. Thus, the data for the 48-hour series is considered to be the most reliable for the calculation of the somatic mutation constant of w^+ to w.

The average mosaic size resulting from irradiation of larvae 48 hours after egg laying is between 7 and 8 facets, and the adult eye contains about 750 facets when development takes place at 25° C. (Margolis and Robertson, 1937). Thus, 48 hours after egg laying approximately 100 loci are present which, following a mutation from w^+ to w, can give rise to a detectable mosaic in the adult eye. Using this estimate of the number of w^+ loci present at the time of irradiation, a somatic mutation constant of about 14×10^{-8} can be calculated for the mutation of w^+ to w.

Reverse Somatic Mutation. Homozygous white-eyed larvae were irradiated 48 and 70 hours after egg laying and the emerging adults inspected for the presence of dark eye color mosaics. In Table 3 the results of these experiments are summarized. No dark mosaics were observed during the inspection of 6,653 white-eyed males and females. The w allele used was spontaneous in origin; w^{48c22} was produced by X-rays; and w^{48G} was induced by sulfur-mustard treatment. A few apricot flies were inspected, the w^a allele probably being of spontaneous origin.

TABLE 3

Incidence of Dark Eye-Color Mosaics Following Irradiation of White Larvae with 1300 r

Allele irradiated	Age at exposure	No. ♂♂ examined	No. ♀♀ examined	No. dark mosaics	No. loci tested	No. loci adjusted to 5000r
w (S)†	48 ± 3 hr.	977	1296	0	700,000*	180,000
w (S)	70 ± 3	788	587	0	780,000	200,000
w^{48c22} (X)	48 ± 3	761	729	0	440,000	115,000
w^{48G} (M)	48 ± 3	527	610	0	350,000	90,000
w^{a} (S?)	48 ± 3	378	0	0	75,000	20,000
Totals	...	3431	3222	0	2,345,000	605,000
Controls	...	1652	0	0	1,300,000	...

*Male larvae irradiated at 48 hours after egg laying are assumed to have 100 white loci per eye anlage; at 70 hours, 200 loci. Female larvae are assumed to have 200 white loci at 48 hours and 400 at 70 hours. Control male larvae are assumed to have 400 testable white loci per eye anlage.

†S signifies spontaneous origin; X signifies X-ray origin; and M signifies sulfur-mustard gas origin.

Only two reports of induction of reverse somatic mutations in *Drosophila* can be found. Timoféeff-Ressovsky (1929) and Patterson (1929) found a total of four dark mosaics in white, eosin, and apricot flies which had been X-rayed in the larval stage. Altogether they examined some 9000 individuals. Two of the mosaic areas were in white-eyed flies, one in an apricot fly, and one in an eosin fly.

The number of white loci tested for reverse somatic mutation can not be determined directly. The estimations of the number of loci present 48 and 70 hours after egg laying, obtained from the direct somatic mutation studies, must be used. It may not be true that the eye disks in the various white stocks develop at the same rate as those in the Canton-S + flies. Thus, the estimate of 100 loci present 48 hours after egg-laying may not be accurate for the white stocks. However, there is no significant difference in the total length of larval life in the + and white stocks. An additional, but reasonable, assumption that somatic cells of females possess twice as many *w* loci as the same

cells in males must be made in order to determine the number of somatic *w* loci exposed to 1300 r in the 48-hour series. For the 70-hour series, by analogy with the direct mutation studies, about 200 *w* loci are assumed to be present at the time of irradiation in each male eye anlage, 400 in each female eye anlage. Finally, the number of somatic *w* loci tested can be reduced to the equivalent of an exposure to 5000 r, on the basis of a linear relationship of somatic mutation to the total dose applied (see Lefevre, 1948a). Thus, in all, approximately 600,000 *w* loci failed to show any evidence of reverse somatic mutation after an exposure equivalent to 5000 r. Admittedly, this estimate of the number of *w* loci tested may be subject to an appreciable error, but in all likelihood the true number tested was not less than 450,000 and may well have been much greater than 600,000.

Discussion

Reverse Mutation. The results of the present study indicate that reverse mutations of various sex-linked recessive genes are not readily induced by X-rays. Following exposure of mature spermatozoa, no evidence of reverse germinal mutation of *y*, *w*, *sn*^{3}, *v*, *m*, or *f* was noted among 166,234 loci irradiated with 5000 r. On the other hand, the same technique of exposure produced nine direct germinal mutations in a total of 43,849 wild-type alleles of these same loci following irradiation with 4250 r.

Previously published data of Timoféeff-Ressovsky (1939) and Johnston and Winchester (1934) would indicate that five to ten reverse mutations might be expected in a sample of 166,234 irradiated recessive loci exposed to 5000 r. The failure to find any in the present study does not of itself signify that reverse mutations can not be produced by X-rays, but suggests that the extra precautions taken to prevent contamination effectively reduced the incidence of flies counted as reverse mutations. In addition to contamination, other effects may mimic induced reverse mutations. A dominant suppressor closely linked to the supposedly reverted locus might not be detected in a routine crossover

test of allelism of the reverted locus and the original recessive locus. Moreover, it is conceivable that a position effect might simulate a reverse mutation. Only Griffen and Stone (1939), among the workers who have reported induced reverse mutations in *Drosophila,* have made salivary chromosome analyses of the irradiated chromosomes to assure their cytological normality. Finally, it must be recognized that spontaneous reverse mutation is not necessarily a very rare event. Rhoades (1931) observed five separate spontaneous reverse mutations of forked during the course of a crossover experiment in which some 42,000 flies were examined.

Considering only the reverse germinal mutation study, the present data point to the possibility that the previous reports of the successful induction of reverse mutations by X-rays may not be wholly reliable. It should be realized that the early workers expected to induce reverse mutations, and they may not have applied especially critical tests in order to rule out other mechanisms that could have been responsible for their results. Moreover, Kaufmann (1942) and Demerec (1938 and personal communication) have also met with failure in extensive efforts to reverse various recessive mutants by irradiation.

However, the evidence against the induction of reverse mutation in *Drosophila* does not rest on germinal mutation studies alone. The experiments reported here include a large-scale test of the X-ray induction of somatic reverse mutation at the white locus. Again, completely negative results were found. An estimated 2,345,000 somatic *w* loci in the larval eye anlagen were exposed to 1300 r, and no dark mosaics indicative of reverse mutation were produced. On the basis of a linear response of mutation to dose, such an experiment is equivalent to the exposure of some 600,000 *w* loci to 5000 r. In the somatic studies white alleles of three diverse origins were used: (a) spontaneous, (b) X-ray induced, and (c) mustard gas induced. It is true that in none of these stocks was a spontaneous reverse mutation observed. Thus, the argument might be advanced that by chance in the experiments yielding negative results the par-

ticular alleles used were incapable of reverse mutation; whereas positive results followed the irradiation of different, but reversible, alleles. Even at the same locus some mutations may be actual losses and others true gene mutations. The findings of Giles, Flynn, and Giles (1948) on the reversibility of various inositol-requiring mutants in *Neurospora* would support such a view. However, the question of possible allelic differences in *Drosophila* can not be settled positively until more data are available.

Patterson (1929) and Timoféeff-Ressovsky (1929) were able to produce dark mosaics in an apricot, an eosin, and a white stock following larval irradiation. Only four such mosaics were found during the examination of 9000 adults. It is difficult to calculate the number of loci irradiated in their experiments, but the total number of somatic loci tested for reverse mutation must have been as great as, or greater than, that in the present study. Two of their mosaics were found in white-eyed flies; of the other two, one was in an apricot and one in an eosin fly. Suppressors are known for both apricot and eosin, so conceivably the latter two mosaics were not true reverse mutations. Furthermore, the two mosaics found in white-eyed flies may well have been of spontaneous origin. In any event, the incidence of the reported induced somatic reverse mutations at the white locus is obviously much lower than the incidence of the reported induced germinal reversals (see Table 1). It should be noted that contamination can not be responsible for any dark mosaics in somatic reverse mutation studies.

At the present time the difficulty in repeating the early work on the X-ray induction of reverse mutation in *Drosophila* throws serious question on the capacity of ionizing radiation to produce true reverse mutation. If such a failure to confirm the earlier reports holds with further experimentation on still other recessive alleles of various origins, it would indicate that the action of ionizing radiation in the production of mutations in *Drosophila*, like that in maize, is essentially destructive.

On the other hand, studies of the induction of reverse

mutations in *Neurospora* appear to indicate that ionizing radiation produces true mutations in that organism. Thus, it is impossible to generalize regarding the mechanism of X-ray induced mutation. However, in the absence of detailed cytological study of *Neurospora* mutants, it can not be claimed with complete assurance that induced reversals represent true gene mutations.

The successful reversion of mutations by irradiation is one evidence that mutations produced by X-rays are qualitatively similar to spontaneous mutations. Even though doubt may now exist that ionizing radiation can cause reverse mutations in *Drosophila*, a different and independent evidence for the similarity of induced and spontaneous mutations has been cited by *Drosophila* workers. Mutations to intermediate alleles are occasionally found following X-radiation. If ionizing radiation is exclusively destructive, only one allele should be produced by irradiation at each gene locus; this presumably being the amorphic allele. Evidence is growing, however, that the gene locus, at least in some cases, is not unitary; but it can be subdivided into smaller regions. As shown by Lewis (1945 and 1948) and Green and Green (1949), these sub-genes interact like alleles, but may be separated by crossing over. If such a condition is general, then even though irradiation is essentially destructive several phenotypically different alleles at a given locus might be produced by X-rays. However, such alleles should be separable by crossing over. Until this possibility is adequately tested, no absolutely irrefutable evidence exists that mutations induced in *Drosophila* by ionizing radiation are identical in kind with spontaneous mutations, rather than being losses of genetic material.

Direct Mutation. The second phase of the present study consisted of a determination of the somatic mutation constant for the X-ray induced mutation of w^+ to w. For comparison, extensive studies of the induced germinal mutation rate have been made by Timoféeff-Ressovsky (1933), Muller (1940), and Bonnier and Lüning (1949), so that a

reasonably accurate estimate of the germinal mutation constant is available. Their data may be combined to indicate a germinal mutation constant for the mutation of w^+ to w of about 12×10^{-8}.

The accuracy of an experimentally determined somatic mutation constant is not at all equivalent to that of a germinal mutation constant. In the first place, a somatic mutation is manifested as a mosaic region in the adult, and no genetic test can be applied in order to assure that a real mutation of the locus in question occurred rather than a mimicking mutation at some other locus, a cell-viable deletion, or a position effect. Secondly, the number of somatic cells originally irradiated must be estimated on the basis of the size of the mosaic area. For eye color mosaics, such an estimate is valid only if each imaginal cell of the eye disk contributes equally (on the average) to adult eye structure.

The w^+ locus is especially favorable for use in determining somatic mutation rates. Of all known eye color mutations in *Drosophila*, only white completely eliminates ommatidial pigment. Thus, any pigmentless mosaic observed in the eye following irradiation of w^+ larvae may safely be classified as an effect at the w^+ locus. Examination of the eyes while the fly is completely immersed in paraffin oil permits an astonishingly accurate separation of mosaics into colorless and pigmented categories.

However, a white mosaic might result if a cell-viable deletion of the w^+ locus occurred, rather than a mutation of w^+ to w. Muller (1935) has shown that very small homozygous deficiencies are not necessarily lethal even to the zygote and, moreover, produce a phenotypic expression characteristic of the amorphic allele of the deleted locus. Stern (1936) has demonstrated that somatic cells can survive with a chromosomal unbalance that would be lethal to a zygote. Fortunately, Demerec (1934) has tested various deletions for cell-lethality. Three different small deletions of the w^+ locus all proved to be cell-lethal. In fact, of several visible loci tested, only deletions at the cut locus were cell-viable; deletions of all other loci were cell-lethal. Thus, it is probably safe to conclude that no significant

number of white mosaics result from deletion, rather than mutation, of the w^+ locus.

The w^+ locus is subject to position effect; the well-known white-mottled conditions result from inversions or translocations which bring the w^+ locus into the proximity of heterochromatin. Among the detected mosaics some may well have been white-mottled rather than mutations. However, in most white-mottled phenotypes the lightest region of the eye is not pigmentless. Further, Muller (1940) has shown that changes to white-mottled are appreciably less frequent than white mutations after exposure to 1000 r, whereas an equal number of each result with a dose of 4000 r. Therefore, among the white mosaics reported in Table 2 some few may have been caused by position effect rather than mutation, but certainly not enough to affect the results significantly. Altogether, the determination of the incidence of somatic mutations of w^+ to w from the frequency of white eye color mosaics may be considered sufficiently accurate to justify a comparison with the germinal mutation rate.

Not only the frequency of white mosaics but also the estimation of the total number of somatic w^+ loci irradiated enters into the calculation of the somatic mutation constant for the mutation of w^+ to w. This estimate can be made by assuming that the fraction of the adult eye occupied by a mosaic reflects the number of somatic loci present at the time of irradiation; *i.e.*, if a white mosaic covers one-eighth of the eye, eight w^+ loci were present at the time of irradiation, one of which mutated to white. That each primordial cell contributes equally to adult eye tissue is difficult to demonstrate. However, if the size range of mosaics produced by irradiation at a given stage in development is relatively small; if mosaics are randomly distributed throughout the eye; if the growth rate of cells carrying induced mutations is not consistently different from that of other cells; if each adult ommatidium is represented at some stage by a single primordial cell; and if relatively few imaginal cells are killed by the irradiation, then the average

mosaic size may fairly be used in order to estimate the number of loci irradiated.

An examination of Fig. 1 shows that irradiation of larvae 48 hours after egg laying results in mosaics showing a normal size distribution in which the mean and modal sizes are very similar. Mosaics occur randomly in all sectors of the eye. No particular mosaic shape is encountered repeatedly. Mosaics extending to the margin of the eye are not significantly different in size from those which are completely internal. It is possible by somatic crossing-over to produce mosaics which simultaneously affect both eye pigmentation and facet structure. In the case of white-split or white-roughest mosaics the same area that is pigmentless also shows the aberrant facet arrangement. Only rarely are facet irregularities observed in the eyes following larval irradiation (see also Patterson, 1929).

All these facts suggest strongly that the primordial cells present at the time of irradiation do contribute equally, on the average, to adult eye structure, and that mosaic size may safely be used to estimate the number of somatic loci irradiated. In Table 2 a somatic mutation constant of 14.2×10^{-8} has been calculated.

The good agreement between this value for the somatic mutation of w^+ to w and the value of 12×10^{-8} for germinal mutation is more than coincidental. The conclusion can be safely made that induced direct mutation of the w^+ locus is independent of the kind of cell in which it is located. In addition, the data warrant the conclusion that induced somatic mutation of eye color loci is independent of the rate of dosage within the limits tested (see Table 2). In conjunction with the data of Lefevre (1948b) using gamma rays, it also appears that induced somatic mutation, like germinal, is independent of wave-length and depends solely on the total dose applied. Thus, radiation-induced somatic mutation is, in all likelihood, identical with radiation-induced germinal mutation.

The use of somatic mutation techniques, where possible, permits mutation experiments on a scale practically impos-

sible in germinal mutation studies. A necessary precaution is that exposures must be made at such a stage in development that the resulting mosaic areas will be neither too large nor too small. It is also desirable to restrict the age spread of the irradiated larvae as much as possible, which becomes successively more difficult at later stages in development.

Conclusions. In the present study no evidence of X-ray induced reverse mutation was found in either germinal or somatic tissue of *Drosophila melanogaster.* However, the direct mutation rate for the mutation of w^+ to w was found to be the same in both germinal and somatic tissue. If direct mutation of a locus is independent of the cellular environment, it is implausible to expect that reverse mutation would be influenced by the kind of tissue in which the gene is located. Admittedly, the weight of the evidence against the induction of reverse mutations lies in the somatic studies reported here. However, this evidence is strengthened by the fact that three different white alleles were tested, as well as by the similarity of the direct mutation rates in germinal and somatic tissue.

In the literature of induced mutation in *Drosophila* reports can be found indicating that little or no difference exists following exposure of spermatozoa and ova (see discussion of Kossikov, 1937). The X-ray sensitivity of spermatozoa in different stages of maturity has also been investigated. Contradictory reports may be found, but a good case may be made for the proposition that induced mutability is similar in all stages (see Timoféeff-Ressovsky, 1937). Induced mutation rates in different species of *Drosophila* are amazingly similar (see Timoféeff-Ressovsky, 1936; Kossikov, 1935; and Christie, 1939). Irradiation over a wide range of temperatures does not affect the mutation rate, except in the vicinity of 0° C. (King, 1947). The only factor that appears to affect the X-ray induced mutation rate significantly is oxygen tension (Baker and Sgourakis, 1950). Oxygen tension appears to have no effect on spontaneous mutation.

In contrast, spontaneous mutability, especially as evidenced by unstable genes, is particularly sensitive to the influence of the cellular environment, sex, age, stage in development, and temperature (see discussions of Demerec, 1931, 1935, and 1941).

In all likelihood the mutations induced by ionizing radiations in *Drosophila,* as in maize, are not qualitatively similar to spontaneous or ultraviolet-induced mutations. Primarily, ionizing radiation is destructive and results in gene loss in both organisms. It follows, then, that mutations induced by ionizing radiation are fixed and incapable of further change by subsequent treatment. The accumulation in a population of a great many such irreversible mutations would have serious evolutionary consequences. At the present time, this view can not be generalized, since in *Neurospora* the evidence indicates that ionizing radiation can produce true gene mutations. Evaluation of the mode of action of all mutagenic agents in various species of plants and animals will be essential to a full understanding of the mechanism by which a gene changes into a new allele.

Summary

Attempts were made to induce reverse mutations in both germinal and somatic tissue of *Drosophila melanogaster.* No evidence of reverse germinal mutation was found following irradiation with 5000 r of some 166,000 recessive X-chromosome loci. In the somatic studies no reverse mutations of white were found in tests equivalent to the exposure of 600,000 white loci to 5000 r. White alleles of three diverse origins were used: (a) spontaneous, (b) X-ray induced, and (c) mustard gas induced. Serious question is thus thrown on the reliability of the early reports of X-ray induced reverse mutation in *Drosophila.*

The rate of X-ray induced direct mutation of w^+ to w was compared following irradiation of germinal and somatic tissue. Data were collected regarding the frequency of germinal mutations to white after exposure of w^+ males, and

an average germinal mutation constant of 12×10^{-8} was determined. From the incidence and average size of white eye color mosaics produced by irradiation of w^+ male larvae, a somatic mutation constant of about 14×10^{-8} was calculated. The possible errors inherent in the estimation of a somatic mutation constant were evaluated. By employing proper techniques, reliable data on the somatic mutation rate of w^+ to w can be obtained. The conclusion was reached that X-ray induced mutability of the w^+ locus is not significantly affected by the kind of cell in which it is located.

A comparison of the influence of various intrinsic and extrinsic factors on X-ray induced and spontaneous mutation indicates that the two mutation processes are qualitatively different. In all likelihood mutations induced by ionizing radiation in *Drosophila*, as in maize, are losses or destructions of genetic material; and unlike spontaneous mutations, induced mutations are incapable of further change.

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ON THE SELECTIVE VALUE OF THE ALLELES *Rt* AND *rt* IN *EPHESTIA KÜHNIELLA*

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INTRODUCTION

THE alleles *Rt* and *rt* in Ephestia influence the pigmentation of the testes. *Rt* testes are brownish purple ("brown"), *rt rt* testes red. (See Caspari, 1933, for colored representations of the two phenotypes.) In breeding experiments, *Rt* turned out to be completely dominant over *rt*. The phenotypic expression of this pair of alleles seems to be rather restricted, as far as its morphological manifestation is concerned; for no other organs containing ommochrome pigments seem to be affected (Caspari, 1943; Wolfram, 1949).

These two alleles seem to occur in wild populations both in North America and in Europe. This polymorphism with respect to testis color was first found in German strains. It was then observed again in American material obtained through the courtesy of Dr. P. W. Whiting. A red-eyed *aa* strain of German origin was found to be homozygous for the gene *rt*, while a *wa wa* (white-eyed) strain recently obtained from Germany through the courtesy of Professor A. Kühn proved to be homozygous for *Rt*. It appears, therefore, that both alleles occur in populations of the species in different continents.

It is generally accepted that in cases of balanced polymorphism a selective advantage for the heterozygote must be postulated (see Fisher, 1930; Ford, 1945). This situation has actually been demonstrated for a large number of chromosome rearrangements in *Drosophila pseudoobscura* (Wright and Dobzhansky, 1946; Dobzhansky, 1947), and

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for a gene affecting body color in *Drosophila polymorpha* (da Cunha, 1949). The present paper deals with the analysis of some factors related to the relative selective advantages of the alleles *Rt* and *rt* in homozygous and heterozygous conditions.

Materials and Methods

The *Rt Rt* and *rt rt* strains employed were the same as those used previously (Caspari, 1943), which had been propagated by brother–sister mating ever since first isolated. They were raised in finger bowls of 4 inches diameter on a diet of yellow corn meal. In every finger bowl offspring from a single female by a single male was raised; 16 F_2 cultures and 20 cultures of backcrosses of the heterozygote to the recessive were observed. The F_2 were raised in a temperature constant room at 18° C., while 18 of the backcrosses had to be transferred at the time of the last larval instar from 18° C. to a room of 24° C.

At hatching, the animals were taken out every morning. The males were dissected and their testis color noted. In this way data for the viability and speed of development for the two phenotypes were obtained. It was furthermore noted which males were found in copulation. In this way a third set of data was obtained which has some bearing on mating behavior.

Results

The results of the experiments are summarized in Table 1. The last column gives the significance of its deviation from expectation. The hypotheses for the expectation were the following: For viability it was assumed that the number of males with brown and red testes would correspond to the Mendelian ratios; for speed of development, equality for the two phenotypes was expected; finally for the number of males copulating, the relative number of males hatching from row 1 was made the basis of the hypothesis.

Viability

The first row indicates that more males with red testes hatched than would be expected. The viability of the

TABLE 1

Viability, Speed of Development and Frequency of Copulation of *Rt*- and *rt rt* Animals in F_2 and Backcrosses

Cross	Phenotype			Significance of difference from expectation (see text)
Rt rt × *Rt rt*	Brown testes	Red testes	Total	
1. Number of males hatching	1,240	471	1,711	$X^2 = 5.8307$ df = 1 P = .016
2. Mean speed of development in days	79.03 ± 0.26	79.94 ± 0.44		T = 1.80 P ~ .07
3. Number of males copulating	139	38	177	$X^2 = 3.2570$ df = 1 P = .07
Rt rt × *rt rt*				
4. Number of males hatching	1,007	925	1,932	$X^2 = 3.4802$ df = 1 P ~ .06
5. Mean speed of development	84.51 ± 0.57	86.26 ± 0.66		T = 2.01 P < .05
6. Number of males copulating	187	124	311	$X^2 = 7.9888$ df = 1 P < .01

homozygous recessive males seems therefore to be somewhat higher than that of the *Rt Rt* and *Rt rt* males combined, and this difference is significant at the 2 per cent. level. In the backcross, this increase in viability of the recessives is not borne out. Actually, there is an insignificant excess of heterozygous males. This result seems to indicate that *Rt rt* males are at least equal and possibly somewhat superior in viability to *rt rt* males. The order of viability of the three genotypes would therefore be expected to be *Rt rt* $\geqq$ *rt rt* > *Rt Rt*. According to the backcross data, *rt rt* males would have a viability of about 0.92, taking the viability of *Rt rt* males as 1. If these values are used to calculate the relative viability of *Rt Rt* males in F_2, a value of 0.46 is obtained. This conclusion is, however, not justified, since the metabolic situation in a culture containing all three genotypes may be different from a culture containing only *Rt rt* and *rt rt* animals. Nevertheless, it seems justified to assume that in F_2 the viability of *Rt Rt* animals is lower than that of *rt rt* males.

In the F_2 data, no excessive variability due to different

numbers per culture was found. The numbers of individuals per culture, including females, varied between 140 and 276. In no instance was heavy crowding apparent. There exists no evidence for heterogeneity between different cultures (heterogeneity $\chi^2 = 15.060$, $df = 15$, $P \sim 0.45$). Furthermore, there is no indication of a correlation between the number of animals per culture and the percentage of "brown testis" males ($r = 0.170$). No systematic investigations on the influence of temperature have been made. Data published in a previous paper (Caspari, 1943) seem to indicate, however, that the excess of *rt rt* males in F_2 is just as pronounced at 24° C. as it is at 18° C. The earlier data are too small to be significant (502 brown : 182 red), but the relative numbers of the two types of males are very similar to those obtained at 18° C. several years later. In the backcross, there was no indication of a difference between the numbers of *Rt rt* and *rt rt* individuals hatching at 24° C. (218 brown : 225 red).

In the backcrosses, there is also no evidence of heterogeneity between the different cultures (heterogeneity, $\chi^2 = 18.238$, $df = 19$, $P \sim 0.5$). But the number of animals hatching per culture was much more variable than in F_2, ranging from 44 to 317 animals. In 8 of the more densely occupied culture dishes, severe crowding and starvation conditions were observed. The hatching animals were small and frequently crippled. An influence of crowding on the differential survival of *Rt rt* and *rt rt* males should therefore show up in this experiment. The percentages of "brown testis" males were plotted against the number of animals hatching from their culture dishes. The results are shown in Fig. 1.

A slight tendency towards association between percentage of brown testis males and crowding in the cultures appears. The relation between the two variables is, however, not simple and straight, since two of the three cultures containing less than 100 animals showed high proportions of *Rt rt* males. The correlation coefficient is consequently not significant ($r = -0.139$). But even if only cultures

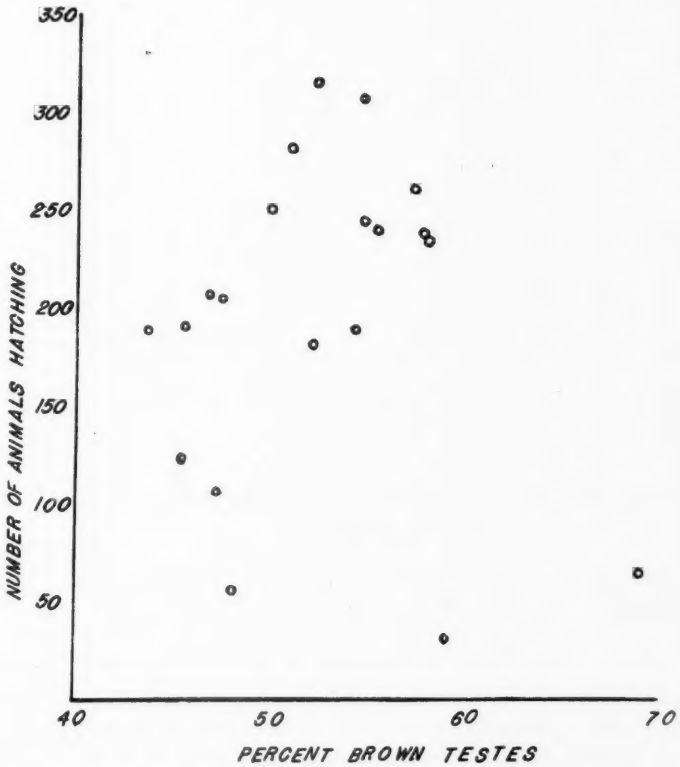

FIG. 1. Scatter diagram showing the relation between crowding and frequency of brown testis males. Abscissa: per cent. brown testis males per culture. Ordinate: number of animals per culture.

with over 100 animals hatching are considered, the correlation coefficient is only $r = +0.409$. This corresponds to a value of $Z = 0.44 \pm 0.26$, well above the 5 per cent. level of significance. It must be concluded that bad conditions such as crowding and starvation do not seem to favor the heterozygote strongly, if at all.

SPEED OF DEVELOPMENT

The data for speed of development of the two phenotypes are shown in Table 1, lines 2 and 5, and in Figs. 2

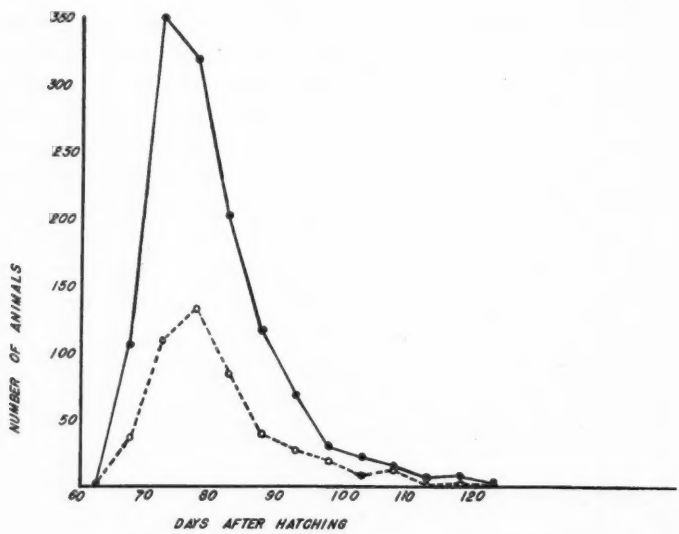

FIG. 2. Speed of development of brown and red testis males in F_2. Abscissa: days after hatching from the egg. Ordinate: number of males hatching. ——— brown testis males. ----- red testis males.

and 3. The difference between speed of development in F_2 and in the backcross is striking and highly significant. This can not be due to the change in temperature at which the backcross was raised, since high temperature results in a speeding up of development. This is actually apparent from the earlier start of hatching in the backcross. The slower mean speed of development in the backcross, on the other hand, is probably due to the crowding and starvation conditions which prevailed in many of the backcross cultures. This is also apparent in the curves of Figs. 2 and 3. While in F_2 the hatching curves show a sharp maximum around 75 days, the backcross shows a flattened curve at about the same rate of hatching extending from 60 to almost 110 days.

In spite of these uncontrolled differences between the two types of cultures, comparison of "brown testis" and "red testis" males gives a consistent picture. In both types of

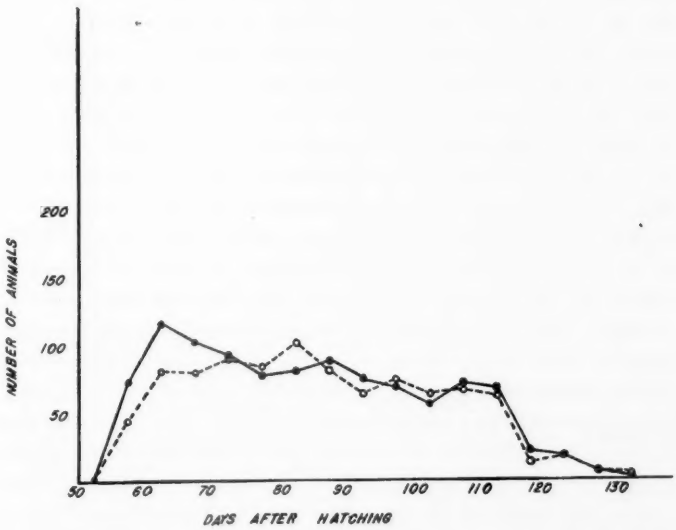

FIG. 3. Speed of development of brown and red testis males in backcross. Abscissa: days after hatching from the egg. Ordinate: number of males hatching. ——— brown testis males. ----- red testis males.

crosses, "red testis" males hatch somewhat more slowly than "brown testis" males. This difference is just at the border of significance at the 5 per cent. level in the backcross ($T = 2.0$, $P = .05$), somewhat above the 5 per cent. level of significance in the backcross ($T = 1.80$, $P \backsim 0.07$). In the backcross, there appears to be no indication that either phenotype is selectively slowed down under bad environmental conditions.

MATING BEHAVIOR

Ephestia usually hatch toward the evening and during the night. They start copulating a short time thereafter, and stay in copula for several hours. Consequently, a considerable percentage of the males was found in copulation on the following morning. It should also be mentioned that Ephestia, both males and females, usually copulate only once during their lives.

If it is assumed that males would copulate at random, it

should be expected that males would be found copulating according to their relative numbers in the cultures. Actually, both in F_2 and in the backcross, more "brown testis" males were found in copula than expected. This difference is insignificant in F_2 ($\chi^2 = 3.2510$, df $= 1$, P $\backsim 0.07$), but highly significant in the backcross ($\chi^2 = 7.9888$, df $= 1$, $P < 0.01$). A difference in mating behavior between *Rt rt* and *rt rt* animals must therefore be accepted.

The mating behavior of *Rt Rt* animals can only be indirectly inferred. In the backcross 17.5 per cent. of the *Rt rt* males and 12.1 per cent. of the *rt rt* males were found copulating. This would give a selective value of 0.692 for *rt rt* males, if the value for *Rt rt* males is taken as 1. Similarly in F_2, 11.4 per cent. "brown testis" males and 8.1 per cent. of the "red testis" males were found in copula. This would result in a selective value for *rt rt* males of 0.710, if the value of *Rt Rt* + *Rt rt* males is taken as 1. The presence of *Rt Rt* males in F_2 therefore does not appear to reduce the selective disadvantage of *rt rt* males due to mating appreciably.

Finding of males in copula may be due to two factors: the frequency of mating, and the time the males spend in copula. Which one of these two factors may be responsible for the observed difference in frequency of males found copulating can not be decided. In Drosophila, it has been shown that the length of copulation may be influenced by the genotypic constitution of the male (Merrell, 1949). On the other hand, genotypic conditions influencing the frequency of mating have been repeatedly observed in Drosophila (*e.g.*, Reed and Reed, 1950).

Discussion

It has been impossible to investigate fully the relative selective advantages of *Rt Rt*, *Rt rt* and *rt rt* animals. This is partly due to the fact that the genes affect a sex-limited character, and that it is therefore impossible to obtain data on the phenotype of the females. It is quite possible that the viability of a certain genetic constitution is quite differ-

ent in males and in females, as has been demonstrated for the SR chromosome rearrangement in *Drosophila pseudoobscura* by Wallace (1948). Furthermore, for the same reason observations on fecundity for the different genotypes are missing.

Finally, the data are incomplete because of the complete dominance of *Rt*, which makes it impossible to differentiate *Rt Rt* and *Rt rt* males in F_2. In this respect, genes of intermediate expression, such as the body color genes in *Drosophila polymorpha* investigated by da Cunha (1949) are certainly preferable. On the other hand, the complete dominance of *Rt* furnishes some material for speculation on the evolutionary origin of dominance, and it was actually with this question in mind that the present investigation was started. In spite of these difficulties, certain conclusions concerning the evolutionary value of the two alleles can be drawn.

Out of six comparisons of "brown testis" and "red testis" males, only two have resulted in differences of a high degree of significance. The advantage in viability of *rt rt* animals in F_2 and the larger number of copulating *Rt rt* males in the backcross are both rather significant. All the other comparisons give differences which should occur by chance only 5–7 times in 100 trials. It is unlikely that all these are chance deviations from the respective hypotheses. Most of them are probably real, but of small numerical value, so that a very large material would be necessary to establish their significance beyond doubt.

As far as speed of development is concerned, the homozygous recessive is slower than the other two genotypes. There is slight evidence for a superiority of the heterozygote over the recessive homozygote, while no evidence is available for the homozygous dominant. On the other hand, there is conclusive evidence that the heterozygote is superior to the recessive homozygote as far as the mating behavior is concerned. At the same time it is at least equal to and possibly superior to the recessive homozygote with respect to viability.

The data do not show beyond doubt the superiority of the heterozygote over both homozygotes in any one of the characters investigated. They do, however, demonstrate that the heterozygote, in every case, is at least equal to and possibly superior to the more favored homozygote. If the less favorable alternative is accepted, equality of the heterozygote with the more favored homozygote, the result may be expressed in the terms that *Rt* is dominant as far as speed of development and copulation behavior are concerned; *rt,* however, is dominant with respect to viability. Similar situations may not be infrequent. Kühn and Henke (1932) have shown for the recessive gene *b* (black wing) in Ephestia that it is dominant in its depressing effect on viability.

The data, though incomplete due to our ignorance of the behavior of the females and of the character of fecundity, make it possible to interpret the occurrence of polymorphism for *Rt* and *rt*. Although heterosis has not been proved for any particular character, there is some indication that it exists. Furthermore, there appears to exist a balance between different pleiotropic effects of the two alleles, *rt rt* being superior in viability but not in speed of development and copulatory behavior. The fact that the heterozygote in every respect is at least equal to the most favored homozygote would tend to give it in practice an advantage over both homozygotes, and in this way would insure the continuing existence of both alleles in natural populations.

Finally, the fact must be discussed that as far as the morphological phenotype is concerned, *Rt* is completely dominant. In itself, this pigmentation character should be completely neutral from an evolutionary point of view, since it is invisible. It must, however, be kept in mind that the importance of pigments is not restricted to their ecological functions, such as visual attraction, warning, protection, and insulation of visual receptors from light. Particularly in holometabolic insects where excretion is restricted during metamorphosis, they constitute a method for removing waste products from the body. This is borne out by the

close chemical similarity of some insect pigments to substances excreted in the urine by vertebrates. The xanthopterin found in Lepidoptera and Hymenoptera has turned out to be identical with the uropterin found in human urine, while the ommochrome pigments of insects are kynurenin derivatives, and therefore analogous to kynurenic acid and xanthurenic acid excreted by mammals.

The testis pigment of Ephestia is skotommin (Becker, 1942). The skotommins in *Rt–* and *rt rt* Ephestia seem to be identical, but the amount is strongly reduced in *rt rt* (Caspari, unpublished). This reduction in the amount of pigment can not be the only difference between the two types of testis pigmentation, since in testes in which the amount of pigment has been reduced strongly, for example, by starvation, brown and red testes can still be easily distinguished. Other chemical differences between *Rt–* and *rt rt* testis pigments must therefore be assumed, possibly in the protein carrier. This is also expressed by the fact that the brown or red color can be distinguished microscopically even in the individual pigment granule. The difference between *Rt–* and *rt rt* pigments appears to be not only quantitative but also qualitative.

It must therefore be assumed that the selective advantages resulting from these testis color genes are of a physiological nature. Fisher (1930*a*) has proposed that in general the more advantageous of two alleles would, by selection of appropriate modifiers, become dominant. He discussed also some cases in which the viability of the dominant homozygote is reduced (Fisher, 1930*b*). In all the cases discussed by him, actually a number of closely linked dominant genes are involved which are at a disadvantage, in homozygous condition, to the universal recessive. While there is no evidence that this latter situation applies to the present Ephestia case, the more general feature is found here: the heterozygote is morphologically not identical with the allele having the higher viability. This situation is explained by Fisher (1930*b*) by the assumption that it would be more advantageous for the heterozygote to resem-

ble the dominant rather than the recessive. This advantage may be ecological, as in the case of the heterostyly genes in Primula (Crosby, 1949), but in most cases it is admittedly of a physiological character.

If this type of reasoning is applied to testis color in Ephestia, it must be concluded that some advantage must be gained for the heterozygote by forming the pigment characteristic of the gene *Rt*. Since the testis color in itself is probably neutral, this advantage must lie in the biochemical processes leading to pigment formation. It may be suggested that the larger amount of kynurenin removed in this way constitutes an advantage, although this is just one out of a great number of possibilities. Of the characters bearing directly on selective advantage, the mating behavior of the males and possibly speed of development behave in the same way as testis color, *Rt rt* being similar to or identical with *Rt Rt*. Viability, on the other hand, is higher in the homozygote recessive than in *Rt Rt* and *Rt rt* combined, and appears therefore to be independent of testis coloration. From this consideration it may be concluded that the type of testis pigment formed is more closely related to the physiological processes resulting in increased number of copulations and possibly higher speed of development than with another pleiotropic effect, viability.

On the other hand, it is quite possible that the dominance of *Rt* has no relation to any selective advantage at all, but is purely the result of developmental conditions (Wright, 1934). It should be mentioned here that if the assumption is true that the pigments in *Rt–* and *rt rt* are qualitatively different, it might be physiologically impossible for the heterozygote to be intermediate with respect to this character. A cell may well be able only to form either brown or red pigment. In this case, complete dominance of one of the two characters would be necessary.

To sum up, the interpretation of polymorphism in Ephestia given here agrees with the experiments of Dobzhansky and his collaborators in demonstrating an overall

selective advantage of the heterozygote over both homozygotes. There is a possible but not significant advantage of the heterozygote in most of the components of selective advantage considered. There is, however, at all events in every component a similarity of the heterozygote with the more favored homozygote, possibly akin to dominance which would add up to an overall heterosis. The finding that the two homozygotes appear to be favored with respect to different pleiotropic effects of selective value would make simple dominance of the advantageous character in the heterozygote sufficient to explain heterosis where one pair of alleles is involved. The complete dominance of the pigmentation characters of *Rt* is assumed to be accidental, and due to either its developmental connection with one of the advantageous characters of *Rt* or to developmental processes not leading to selective advantage.

Summary

Viability, speed of development and mating behavior were studied in F_2 and backcrosses involving the testis color genes *Rt* and *rt* in Ephestia. In F_2, there is a significant excess of recessives, while in the backcross the heterozygote has an insignificant advantage. *Rt rt* males are significantly more frequently found in copula than *rt rt* males. Finally, the development of *Rt Rt* and *Rt rt* is slightly but insignificantly faster than of *rt rt* males.

It is concluded that each one of the two homozygotes may be favored in some components of selective advantage. The heterozygote is in all cases at least equal to and possibly superior to the more favored homozygote. This would add up to a selective advantage of the heterozygote over both homozygotes, a condition necessary for the establishment of balanced polymorphism. The dominance of *Rt–* with respect to the pigment produced is considered to be accidental.

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SEMI-ALLELIC GENES[1]

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While working on the interrelation of the closely linked genes miniature and dusky in *Drosophila virilis* some years ago, I was impressed by the frequent occurrence of gene groups like this in various animals and man. Even the blood-group genes A–B were suspected of belonging to this category.[2] This idea has since haunted me, and I have been gathering data concerning this question. Recently, some data apparently favoring such a view came to my notice. Also, opinions similar to mine have been presented by some blood-type specialists (*e.g.*, Race, 1949). Thus I have been encouraged to publish this short paper.

There are many cases where two or more genes of similar phenotypic effect are located in close sequence on the chromosome. This kind of gene group has been called "step-allelic" (Dubinin, 1929), "partially allelic" (Agol, 1930), "pseudo-allelic" (Lewis, 1947), "semi-allelic" (Muller, 1949), or "twin" genes (Komai, 1947). Such gene groups may be found not only in Drosophila, but also in various animals as well as in man. In Drosophila they are considered to have their chromosomal basis in the so-called repeats of bands (Bridges, 1938). I shall use in this paper the term "semi-allelic" genes, proposed by Muller to designate them.

The Common Features of Semi-Allelic Genes

1. These genes are located in direct sequence on the chromosome, without any other intercalated gene, and crossing over, if any, very rarely occurs between them.

[1] Contributions from the National Institute of Genetics, Japan. No. 2.

[2] Komai and Takaku (1949). The manuscript of this paper was sent in 1944 to the editor of *Cytologia*, but was not printed until 1949.

2. The phenotypes are similar, but not quite identical, and the difference is often qualitative, not merely quantitative.

3. A covering effect is commonly seen in their heterozygote, namely, the heterozygote approaches the wild-type more than does either homozygote.

4. The so-called mosaic inheritance is seen, when both genes are dominant.

It is generally hard to distinguish the semi-allelic genes from the allelic genes in the strict sense, when the recombination of the genes is not ascertained. Even in such cases, the discrimination is often possible, by the features 2, 3, and 4 enumerated above, since in most allelic genes the difference is quantitative, and the heterozygote shows either an intermediate phenotype, or it is nearly identical to one of the homozygotes (Stern, 1930; Goldschmidt, 1938). The semi-allelic genes may be distinguished from the ordinary linked genes by the similarity of their phenotypes and by the complete or nearly complete absence of recombination.

As a representative of the semi-allelic gene group, I shall take miniature (*m*) and dusky (*dy*) found in Drosophila. These genes give very similar phenotypes, namely, the wings are considerably shorter and narrower than the normal wings, and the surface appears distinctly darker due to the smallness of individual cells and crowded hairs. It is rather hard to distinguish them from each other by their phenotypes only, especially when their lower-degree alleles are compared. Generally speaking, however, miniature has smaller wings and dusky has darker wing surface. This gene pair is found in *D. melanogaster, D. virilis, D. pseudoobscura* and *D. ananassae,* in exactly identical relation, *viz.,* they are located on the first chromosome in direct sequence, so close to each other that scarcely any crossing over takes place between them. Takaku and I have worked on the phenotypes of various alleles in various combinations of these genes in *D. virilis* (Komai and Takaku, 1949). We have found that they are not real recessive genes, as com-

monly assumed, because the heterozygotes can be separated from the wild-type by the difference in the proportional length of wing parts, and that *m/dy* differs from the wild-type to a smaller degree than does either *m/m* or *dy/dy* from the latter. Crossing over actually takes place between the genes in this species, though the frequency is as low as 1/1000–1/3000. Thus, although in other species scarcely any crossing over has been confirmed to occur between them, the non-allelism of *m* and *dy* is beyond question.

Semi-Allelic Genes in *Drosophila melanogaster*

The semi-allelic genes found in *D. melanogaster* are listed by Grüneberg (1937). The new list presented in Table 1 has been compiled by adding to his list some more recently discovered ones selected from Bridges and Brehme's monograph (1944).

Semi-Allelic Genes in Other Insects

The pattern genes in the grouse locusts worked out by Nabours and his associates afford apparently good examples of semi-allelic genes. These genes are dominant, and show mosaic inheritance. Many of them behave like alleles, but some show independent assortment, or linkage relation. A few of the latter recombine with a very low frequency. In *Paratettix texanus* " twenty-one genes are so closely linked, or approach multiple allelism to such an extent, that none has crossed over with any other. One, *Hm*, actually crossed over with members of this group five times in over 7000 pairings " (Nabours, 1930, p. 351). In *Apotettix eurycephalus* genes *O, R, W, Z* form a group much like an allelic one, gene *T* lies 0.5 unit apart from this group, while *G* and *K* are located so closely to *T* that there is only 0.3 unit distance. In *Telmatettix aztecus* four pattern genes behave as an allelic group (Nabours, 1929).

The pattern genes of the variable lady-beetle *Harmonia axyridis* seem to be in the same interrelationship as the pattern genes of the grouse locusts just mentioned. Hosino (1936–1948, 1940) has been able to discriminate more than

TABLE I

Obvious and Probable Semi-allelic Genes in *Drosophila melanogaster* Selected from Bridges and Brehme's Monograph (1944)

Gene	Symbol	Chromosome	Locus	Phenotype
chlorotic	*cc*	I	-0.1	Body greenish yellow
yellow	*y*	I	0.0	Body and bristles yellow
silver	*svr*	I	0.0 ±	Body and legs pale, bristles dark
pallid	*pl*	I	0.0 ± I	Body and wings pale
achaete	*ac*	I	0.0 +	Post-dorsocentrals gone, hairs fewer
scute 1-	*sc*	I	0.0 +	Loss of bristles in definite pattern
Abruptex	*Ax*	I	3 ±	L5 and other veins shortened, wings shortened, arched
Confluens	*Co*	I	3 ±	Veins thickened especially toward tips, $Co/Ax = Ax/+$
lozenge	*lz*	I	27.7	Eyes narrow, ovoid
almondex	*amx*	I	27.7 ±	Eyes slightly reduced, narrow below, lz/amx = +, c.o. occurs between lz^s and lz^g
raspberry	*ras*	I	32.8	Eye color dark ruby
vermilion	*v*	I	33.0	Eye color bright vermilion
miniature	*m*	I	36.1	Wings small, surface dark gray
dusky	*dy*	I	36.2	Wings small, surface dusky
Star	*S*	II	1.3	Eyes small, rough
asteroid	*ast*	II	1.3 +	Eyes small, rough
bright	*bri*	II	54.3 ±	Eye color bright red
purple	*pr*	II	54.5	Eye color purplish ruby
light	*lt*	II	55.0 -	Eye color yellowish pink
lightoid	*ltd*	II	56.0 ±	Eye color like *lt*, but lighter, ltd/lt = +
blot	*blt*	II	55.2 ±	Wings inflated, with plexus
puff	*puf*	II	58.0 ± 2	Wings blistered
bloated	*blo*	II	58.5	Wings ballooned, extra veins
arch	*arch*	II	60.5 ±	Wings down-curved
archoid	*ad*	II	60.7	Wings arched, broad, short
blistered	*bs*	II	107.3	Wings blistered, small, extra veins
balloon	*ba*	II	107.4	Wings inflated, extra veins
roughoid	*ru*	III	0.0	Eyes small, rough, facets erupted
Roughened	*R*	III	1.4	Eyes rough, facets large

TABLE I—*continued*

Gene	Symbol	Chromosome	Locus	Phenotype
maroon	*ma*	III	49.7	Eye color dull ruby
rosy	*ry*	III	51.0 ±	Eye color deep ruby
karmoisin	*kar*	III	52.0	Eye color dull scarlet
stubbloid	*sbd*	III	58.2	Bristles condensed, wings shortened
Stubble	*Sb*	III	58.2	Bristles much condensed, wings shortened
bithorax	*bx*	III	58.8	Metathorax mesothorax-like, balancers enlarged
bithoraxoid	*bxd*	III	58.8	Metathorax somewhat mesothorax-like, balancers disc-shaped
glass	*gl*	III	63.1	Eye color dilute, facets fused
glasslike	*gl-l*	III	64.0 ±	Eye color orange, eyes rough, small
cardinal	*cd*	III	75.7	Eye color dull scarlet, ocelli white
white ocelli	*wo*	III	76.2	Ocelli colorless

twenty different genes governing the pattern type of this insect which behave as alleles. When individuals having different patterns are mated, both parental characters usually appear in the heterozygote like a mosaic (Tan, 1946).

In the silkworm about 150 mutant genes referable to 16 linkage groups have been discovered (Takami, 1948). Of these there are three cases of probable semi-allelic gene groups:

1. *Ma–p–S2*: Chromosome II genes controlling larval pattern. *Ma*—pattern characteristic of *Theophila mandarina*, the alleged ancestor of the silkworm; *p*—the commonest larval patterns comprising eight different types apparently allelic; *S2*—striped pattern; the distance: *Ma–p* = 1.8 units, *p–S2* = 1.4 units.

2. E–E^{Kp}–E^{Co}–E^{El}–E^{D}–E^{H}–E^{Nc}–E^{N}–E^{Cl}. Nine genes located at 0.0 of chromosome VIII. These are in similar interrelation to the brachyury gene group in the mouse to be mentioned later. They behave as if they were either

allelic or tightly linked genes. All produce extra legs or extra semi-lunar patterns on the first two or three abdominal segments of the larva; a few (E, E^{Nc}) efface the ordinary semi-lunar patterns; some (E^{Kp}) have a lethal effect also. Each gene possesses its peculiar phenotypic effect, which is covered in the heterozygous combination of at least some of them. Also there have been observed some cases of recombination between two of them (E^{Kp} and E^{N}). Another gene, *Nc*, located 1.4 unit apart, produces the same kind of abnormality as the genes just mentioned, effacing the ordinary semi-lunar patterns, beside having recessive lethal effect.

3. *ae–be:* a pair of genes located on chromosome IX, cause deficiency of amylase, *ae* that in the stomach, *be* that in the blood: locus 0.0 and 1.1, respectively.

In the Tortricid moth, *Acalla* (*Peronea*) *comariana*, according to Freyer (1928), the high variability of the wing pattern is due to a group of allelic genes, B, B^1, B^2. In addition, there is a gene C producing a costal blotch on the fore wing. This gene is so closely linked with B that no recombination was found except a single doubtful case.

The " complementary " sex genes, $X_a \ldots X_t$, in the parasitic wasp, *Habrobracon juglandis*, according to Whiting (1943, 1946), also seem to represent a case of the semi-allelic gene group. Different strains have different sex genes which produce males in the hemizygous or homozygous state, while in the heterozygous state their efficiency is lowered, and females result. Inaba (1944) proposed a practically identical hypothesis on the determination of sex in this wasp, without knowing of Whiting's theory.

Semi-Allelic Genes in Vertebrate Animals

In the tropical fish, *Lebistes reticulatus*, a number of genes producing color patterns show mosaic inheritance. Of these, nine are closely linked to the male sex factor and three to the female sex factor. A single case of recombination was observed between *ti* and *lu* belonging to the latter group (Winge, 1927).

In the muscovy duck, *Cairinia moschata,* two antigenic complexes A_1 and A_2 have been discovered. Both include three or four subtypes whose genes are in the interrelation characteristic of semi-allelic gene groups (McGibbon, 1946).

The famous gene group causing skeletal abnormalities in the mouse's tail, T–t^0–t^1–*Fu*–*Ki*, is undoubtedly a typical example of this sort. Thus, T/T, t^0/t^0 and t^1/t^1 are lethal, while T/t^0, T/t^1 and t^0/t^1 are viable; recombination occurs between T and *Fu*, or between T and *Ki* with frequencies from 2 to 5 per cent. (Dunn and Gluecksohn-Schoenheimer, 1942; Dunn and Caspari, 1945).

Among some thirty different antigenic genes thus far discovered in cattle, *B*, *G*, and *K* are the most interesting from the genetic viewpoint. "There are genetically two kinds of animals possessing *B* and *G* together—namely, (*a*) those in whose offspring *B* and *G* separate as expected if the parents were heterozygotes, (*b*) those whose offspring either contain both *B* and *G* or neither. Seemingly, there is a linkage of the causative genes of these three substances or they are produced by genes in an allelic series. Thus, under the second explanation, one gene would produce *B*, another *G*, another *BG* together and another *BGK*" (Irwin, 1947, p. 154). This seemingly enigmatic interrelation of the genes *B*, *G*, *K* can be accounted for by assuming that they belong to a semi-allelic gene group.

The rabbit possesses blood type genes H_1 and H_2 which are apparently in the same interrelation as the human *A* and *B* blood-group genes (Plate, 1938).

Semi-Allelic Genes in Man

To the writer the human blood-type gene sets, *A*–*B*, *M*–*N*, and Rh^0–*Rh'*–*Rh''*, also seem to belong to the semi-allelic genes. Of these, the *A*–*B* gene set has all the characteristics of semi-allelic genes enumerated above, namely, they produce similar, but not identical, antigens; they are dominant genes, and the mosaic inheritance and covering effect are shown in the heterozygote *AB*. The question as to whether they are allelic genes or not has been discussed much among the previous authors, and the opinion is now

perfectly settled in favor of the allelism theory. In Wiener's handbook, for instance, we can see very clear and thorough presentations of this matter. Thus, the other theories, notably, von Dungern and Hirzfeld's theory assuming the location of these genes on different chromosome pairs, and Bauer's theory postulating their linkage on the same chromosome pair, now seem to have been completely excluded. In spite of this, there are facts which do not quite agree with the allelism theory. Especially, there are a few fairly certain "Bernstein exceptions" where an *O* child was born of an *AB* mother (Haselhorst and Lauer's case, Kossovitch's case), or an A_1B child was born of an $A_1B \times A_2$ couple (Dahr and Bussman's case, all from Wiener, 1943). These exceptions are sometimes attributed to mutation or to nondisjunction of the chromosomes carrying one *A* and the other *B* factor, but they can as well be accounted for by assuming an exceptional recombination of these genes. Bauer's theory postulating a linkage between *A* and *B* (1929) was severely criticized by Bernstein (1930) on mathematical grounds. This author has shown conclusively that, if crossing over occurs between *A* and *B*, however low its frequency might be, it will eventually bring about a situation very different from the actual state. This argument is undoubtedly convinc on the whole; still it does not seem to have thoroughly disproved the semi-allelic interrelation of *A* and *B*, and the possibility of very rare, rather exceptional, occurrence of recombination, especially because the argument is based on purely theoretical grounds postulating a random mating among an unlimited number of people for a great number of generations, and also the survival and propagation of all recombination individuals. The same author also contends on *a priori* grounds that the coincident occurrence of similar genes in direct sequence is highly unlikely. This contention is unwarranted, in view of the frequent occurrence of semi-allelic gene groups.

Furuhata (1927, 1929) assumes an absolute linkage of genes $A(a)$ and $B(b)$. For this his theory is generally considered to be essentially identical with the allelism theory. Stated more accurately, it is rather the semi-allelism theory.

As to the blood-type genes, *M–N*, both family data and statistical data strongly support their allelism. Recently, however, another cellular antigen *S* belonging to the same series was discovered. This antigen shows positive reaction with 72 per cent. *M*, 60 per cent. *MN* and 33 per cent. *N*, and its gene *S* occasionally recombines with either the gene *M* or *N* (Sanger, 1949). Thus, probably *S* and *M–N*, possibly all three, form a semi-allelic group.

For the interrelation of the three subtypes of the *Rh* antigen, much discussion is being exchanged between Fisher and his associates on the one side and Wiener and his associates on the other, the former maintaining the linkage theory and the latter the allelism theory. Race (1949) expressed on this question an opinion which is essentially a semi-allelism theory, that *Rh*^{0}, *Rh'* and *Rh''* are closely linked genes resembling triple-allelic genes.

Among the human pathological genes, the most probable semi-allelic group is that of the color-blindness, protanopy and deuteranopy. A few pedigrees, including both the subtypes, have been published. The non-allelism of the genes concerned is shown by the normality of the individual who possesses these genes in the recessive state (Komai, 1934, 1947). The two subtypes of *retinitis pigmentosa*, dominant and recessive, perhaps represent another human case of a semi-allelic gene group. Haldane (1936, 1941) locates these genes at 28 on the homologous section of the X-chromosome.

Discussion

The list of the semi-allelic gene groups presented above is far from comprehensive. Still, we are impressed by the frequency and diversity of their examples, and their presence in various groups of animals including man. This kind of genes goes insensibly over to the real allelic genes on the one side, and to the linked genes on the other. So it is always possible that a group of genes which has been considered to be real allelic genes may be shown to be an example of semi-allelic gene group, or *vice versa*.

The semi-allelic genes are often considered to have their

chromosomal basis in "repeats," as mentioned above. However, some may be located within a very short length—even within a single band (*e.g.*, several *scute* genes). Others are undoubtedly combined with a sectional change of the chromosome (*e.g.*, some *scute* genes, and the brachyury genes of the mouse). Thus the semi-allelic genes probably include many different cases as to their chromosomal bases. Also it is conceivable that there are chromosomal sections of fairly large length, corresponding to several ordinary gene lengths, having nearly uniform nature throughout, and a change affecting any part of this section will produce similar phenotypic effects. It is also probable that semi-allelic genes originate as duplicates of a single gene, and that they are more tightly bound together than genes of independent origin, so that recombination between them, if any, takes place very rarely, as compared with that between genes of different nature. At any rate, the frequent occurrence and wide distribution of this kind of gene groups merits special attention.

Summary

1. The semi-allelic genes are a group of genes with similar phenotypic effect, located on the chromosome in direct sequence, and crossing over, if any, occurs between them with very low frequency. They may be distinguished from the true allelic genes by the mosaic appearance of the characters and the covering effect in the heterozygote. They differ from the ordinary linked genes in the similarity of their phenotypes and in the complete or nearly complete absence of recombination.

2. The semi-allelic genes are rather common, and widely distributed in various animals and man. Many examples are presented from among mutant genes in Drosophila, other insects, vertebrate animals and in man.

3. It is possible that *A–B*, *M–N–S*, and Rh^0–*Rh′*–*Rh″* blood-type genes also belong to the semi-allelic group.

Addendum

After this paper had gone to press, the writer came to know through the kindness of Dr. C. Stern that Gold-

schmidt, 1944, pp. 183–210, gave several instances of crowding of mutants of similar phenotypic effect in the same region of the chromosome in *Drosophila*. The reader is referred especially to Chapters VI and VII of this paper. Goldschmidt gives evidence for these "genes" being in reality due to rearrangement effects.—T. K.

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A SEARCH FOR SOME EFFECTS OF THE "SHORT-EAR" GENE ON BEHAVIOR IN MICE[1]

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INTRODUCTION

DIFFERENCES in behavior between animals of the same or different breeds have often been observed. If the difference is exhibited in a constant environment, it is assumed to have a genetic cause. The exact genetic basis for behavioral differences has, with a few notable exceptions, such as *waltzer* in the house mouse, been difficult to determine.

The genetics of behavior may be studied in either of two ways. First, after a difference in behavior, not due to varying environments, has been observed, it may be analyzed by the usual genetic method of making crosses between the two or more kinds of animals. This approach has the disadvantage that observable behavioral differences may have a complex genetic basis and so be difficult to analyze. Second, two groups of animals that are known to differ by a single gene may be tested for a wide variety of behavioral differences. This approach is obviously not free of disadvantages, but does offer the possibility of a clear-cut genetic basis for any behavioral difference discovered.

The present experiment is a search for effects on some simple types of behavior of the "short-ear" gene (*se*—autosomal recessive) in the house mouse, *Mus musculus*. It is a repetition with some refinements of a previous experiment (Bundy, 1948). The following behavioral characteristics were measured: (1) shock avoidance in a simple maze, (2) conditioning to a pure tone, (3) hearing intensity threshold, (4) high frequency hearing threshold, and (5)

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urination during the shock avoidance testing and the threshold probing.

With the exception of hearing intensity threshold, no difference in behavior was found.

Materials and Methods

MICE

Twelve pairs of mice were used in this experiment. The mice of each pair were the same sex from the same litter, one member of a pair being normal-eared (*Sese*) and the other short-eared (*sese*). Seven pairs were females and five pairs were males. They were treated as much alike as possible. The members of each pair were kept in the same cage, fed the same food, and testing within a ten-minute interval of each other.

The mice were all descendants of a single pair in the twenty-fifth generation of a strain designated as *Sese-Ab2,* in which all matings have been brother by sister of the type *Sese* by *sese*. The members of a pair are, as a consequence of this breeding plan, alike for all or nearly all genes except for the one pair deliberately kept heterozygous. The original pair was obtained from Dr. E. L. Green, of the Ohio State University. The origin of the strain is described in a paper by Green and Green (1946).

APPARATUS

Testing cage. The testing cage measured 15 inches long by 10 inches wide by 7 inches deep. The floor was covered by parallel bare copper wires about one-fourth inch apart and was divided into two sections by painting one half white and the other half black. In each section of the floor alternate wires were soldered to a lengthwise wire. With this arrangement it was possible to apply a voltage to the grid on one half while the other remained uncharged. The walls were painted to match the floor, giving a clear mark where the uncharged grid ended and the charged grid began. A flat piece of glass was placed over the top.

Sound source equipment. A buzzer was hung inside the box on wires so that it did not come in contact with the wall. It was operated by dry cells.

Earphones were also hung in the cage on the same wires at a later time. The source of oscillating power was a Hewlett-Packard audio oscillator, Model 200B. With the use of dials the frequency and amplitude of the tone emitted from the earphones could be varied. The amplitude was measured in per cent. of the maximum output.

Electrical switch. A manually operated two-pole double-throw switch was used. The switch operated as follows: In down position to one side, voltage was applied only to the left side of the cage. In the upright position, auxiliary wires attached closed the circuit for either the buzzer or the earphones, whichever one was being used. In the upright position, neither side was charged. In the down position to the other side, only the right side of the box was charged.

CRITERIA

(*1*) *Shock avoidance in a simple maze.* A mouse was placed on the uncharged side of the testing cage. After it became completely still, the switch was moved to the upright position completing the buzzer circuit. Since most of the mice cringed when first hearing the buzzer, it was not considered a conditioned response. A second later, with some human error, the switch was thrown on down completing the grid circuit on the side on which the mouse had been placed. Thus the mouse should move to the other side of the cage upon hearing the buzzer.

It is conceivable that occasionally a mouse would have jumped or given other indication of hearing at the exact instant the buzzer was sounded, independently of whether the buzzer sounded or not. This is a source of error but may be cancelled out due to the fact that the experiment was a comparison of the two types of mice and that it is not likely that any more error of this kind was introduced by one type of mouse than by the other.

Each mouse was given five trials a day for thirteen days, or a total of sixty-five trials.

Positive responses were divided into two categories: (1) if a mouse suddenly jumped, cringed, or turned its head at the sound of the buzzer, it was concluded that the mouse heard the sound; (2) if the mouse got completely to the other side, he thus avoided the shock.

(*2*) *Conditioning to a pure tone.* In this part of the experiment, the dry cells were replaced with the audio oscillator and the buzzer was replaced by the earphones. The frequency was adjusted to 1,000 cycles and the amplitude was set at 100 per cent. The same mice used before were then individually tested in the same manner except that a pure tone was used instead of the buzzer and they were given ten trials per day instead of five. They were given these daily until they demonstrated positive response to at least 80 per cent. of the trials for two consecutive days. The mice were then said to be conditioned to move upon hearing the tone. A positive response was either complete shock avoidance or other indication of hearing.

(*3*) *Hearing intensity threshold.* After the mice had been conditioned to the pure tone, they were subjected to the tests to determine the hearing threshold.

Each mouse was given two trials a day for ten days at 80 per cent., 60 per cent., 40 per cent., 20 per cent., and 10 per cent. of the maximum amplitude at 1,000 cycles. The sequence of amplitudes in each day was picked at random.

A record was made of the positive responses at each amplitude. These were totalled; a perfect score was therefore twenty for each amplitude. The number of positive responses was plotted along the ordinate, and the amplitude along the abscissa. For each mouse an eye-fitted curve was drawn. The hearing threshold is the amplitude (expressed in per cent. of maximum volume) at which the mouse responded 50 per cent. of the time. The X value corresponding to $Y = 10$ was recorded for each mouse.

(*4*) *High frequency hearing threshold.* The same mice were given two trials per day for ten days at 2,500 cycles,

5,000 cycles, 10,000 cycles, 15,000 cycles and 20,000 cycles with the amplitude set at 100 per cent. The sequence of the daily trials was picked at random. Again a perfect score was twenty for each frequency. As in the case of intensity threshold curves were constructed from the data on each mouse. The X value corresponding to $Y = 10$ was recorded.

(*5*) *Urination during shock avoidance and threshold probing.* A record was made of urination at any time in the testing cage during the preliminary buzzer testing, or during the threshold probing. Since each mouse was in the testing cage thirteen days in the buzzer testing and twenty during the threshold probing, the highest score possible was thirty-three.

STATISTICAL ANALYSIS

For each of the measured behavioral characteristics, a t-test for paired comparisons was used (Snedecor, 1946). "$\bar{x}$" is the mean difference of the sample. "P" is the probability of getting larger values of "t" by chance alone, provided there is no difference between the two types of mice.

RESULTS

(*1*) *Shock avoidance.* All the mice indicated hearing the buzzer, so no analysis was necessary. In seven of the twelve pairs, the normal-eared mice avoided the shock more times than the short-eared mice. In the remaining five pairs, the short-eared mice avoided the shock more often. The mean difference $\bar{x} = 4.42$, $t = 1.19$ and $P = 0.3$–0.2. It was concluded that the normal- and short-eared mice were not different in ability to avoid the shock.

(*2*) *Conditioning to a pure tone.* The analysis indicates that there was no behavioral difference between normal- and short-eared mice with respect to conditioning to a pure tone of 1,000 cycles. The $\bar{x} = 0.33$; $t = 0.62$; $P = 1.0$–0.5. Fewer days were required to condition the normal-eared mice in three pairs, while the short-eared mice were better in six pairs. Three pairs were tied.

(*3*) *Hearing intensity threshold.* There is significant evidence against the hypothesis that the two types of mice are the same with respect to intensity threshold. The $\bar{x} = 4$; $t = 2.68$; $P = .05–.02$. Of the twelve pairs of mice, all except one indicate a hearing advantage in favor of the normal-eared mice. It is therefore concluded that the short-eared mice have a higher hearing intensity threshold.

(*4*) *High frequency threshold.* No difference was found, between the two types of mice in high frequency threshold. The $\bar{x} = 1.54$; $t = 1.79$; $P = 0.2–0.1$. In ten of the twelve pairs, the normal-eared mice heard higher frequencies. The analysis, however, did not show evidence against the hypothesis that the two types of mice are the same. We could expect by chance alone larger values of t between twenty and ten per cent. of the time.

(*5*) *Urination.* There was no difference in urination between the two types of mice when electrically shocked. The $\bar{x} = 1.25$; $t = 1.17$; and $P = 0.3–0.2$. The normal-eared mice urinated more often in eight of the pairs while the short-eared mice urinated more often in the remaining four pairs.

Discussion and Summary

With the exception of hearing intensity threshold, no difference in behavior between normal- and short-eared mice was found with respect to (1) shock avoidance in a simple maze, (2) conditioning to a pure tone, (3) high frequency hearing threshold, and (4) urination. It was concluded from the analysis that the normal-eared mice have a lower hearing intensity threshold, *i.e.*, the normal-eared mice can hear sounds of lower intensity than can the short-eared mice.

The results of the preliminary experiment (Bundy, 1948) were the same as those reported here with the exception of high frequency hearing threshold which was not done before. Ten pairs of mice were used in the preliminary experiment. In eight of the ten pairs, the normal-eared mice had a lower intensity hearing threshold. Combining

the data from both experiments, we find that of the twenty-two pairs of mice, the normal-eared mice had a hearing advantage in nineteen pairs.

This superior ability of the normal-eared mice to hear sounds of lower intensity may be due to the larger pinna or to a difference in internal ear structure. It would be of value to do an experiment using normal-eared mice with the pinna clipped to the same size as the short-eared mice. If the larger pinna is the source of better hearing, then this advantage would be removed.

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